



BACTERIOLOGY

CONTENT

1. Structure of bacterial cell

- Cell wall
- Outer membrane
- Glycocalyx
- Plasma membrane
- Cytoplasm
- Surface appendages
- Endospores

2. Bacterial genome

- Horizontal gene transfer and genetic recombination
- Interrupted mating experiment

3. Nutritional types of bacteria

- Culture media for bacterial growth
- Bacterial growth
- Measurement of bacterial growth
- Control of microbial growth

4. Staining

- Gram staining
- Acid fast staining

5. Bergey's manual of systematic bacteriology

6. C R Woese's 3 domain system

Structure of bacterial cell

Size:

- 0.2- 2 micrometers in diameter
- 0.5-5.0 micrometers in length
- Few examples like, ***Thiomargarita namibiensis*** and ***Epulopiscium fishelsoni***- very large and visible to unaided eye
- Smallest bacteria, ***Mycoplasma*** (0.3 micrometer diameter)

Shape: 4 basic shapes of bacteria:

- **Bacillus** (rod like)
- **Coccus** (spherical or ovoid)
- **Vibrio** (comma shape)
- **Spirilla** (spiral or helical shaped)

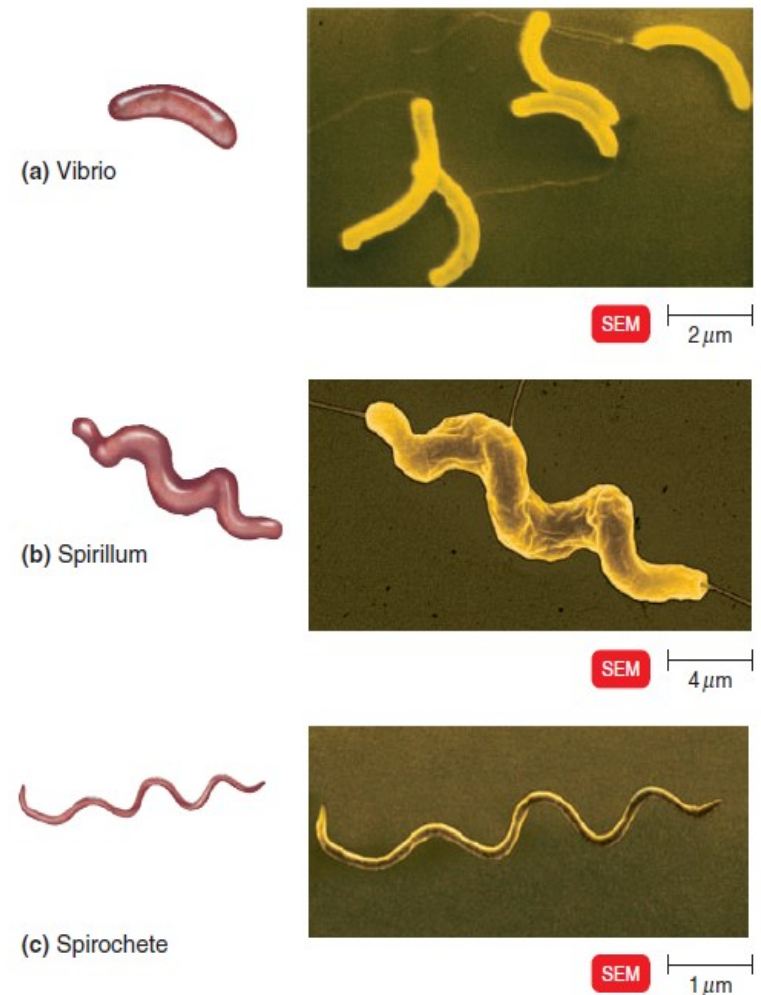
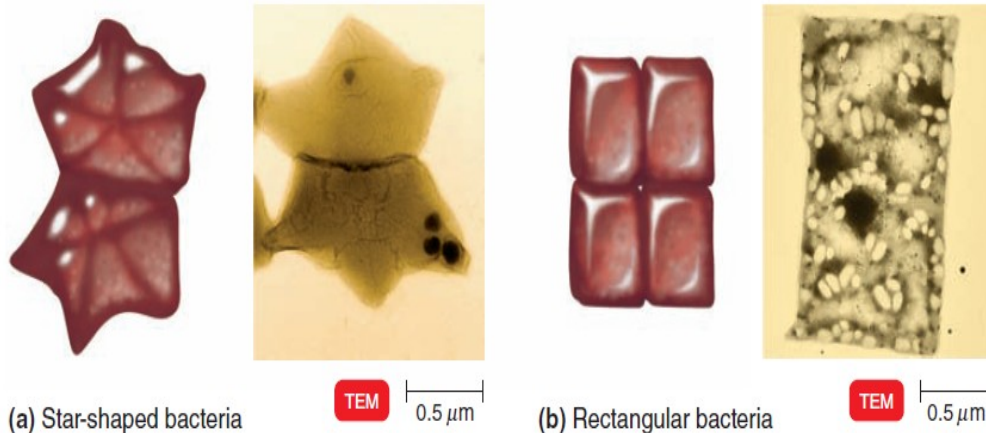


Figure 4.4 Spiral bacteria. (a) Vibrios. (b) Spirillum. (c) Spirochete.

Figure 4.5 Star-shaped and rectangular prokaryotes. (a) *Stella* (star-shaped).

(b) *Haloarcula*, a genus of halophilic archaea (rectangular cells).

Q What are the common bacterial shapes?

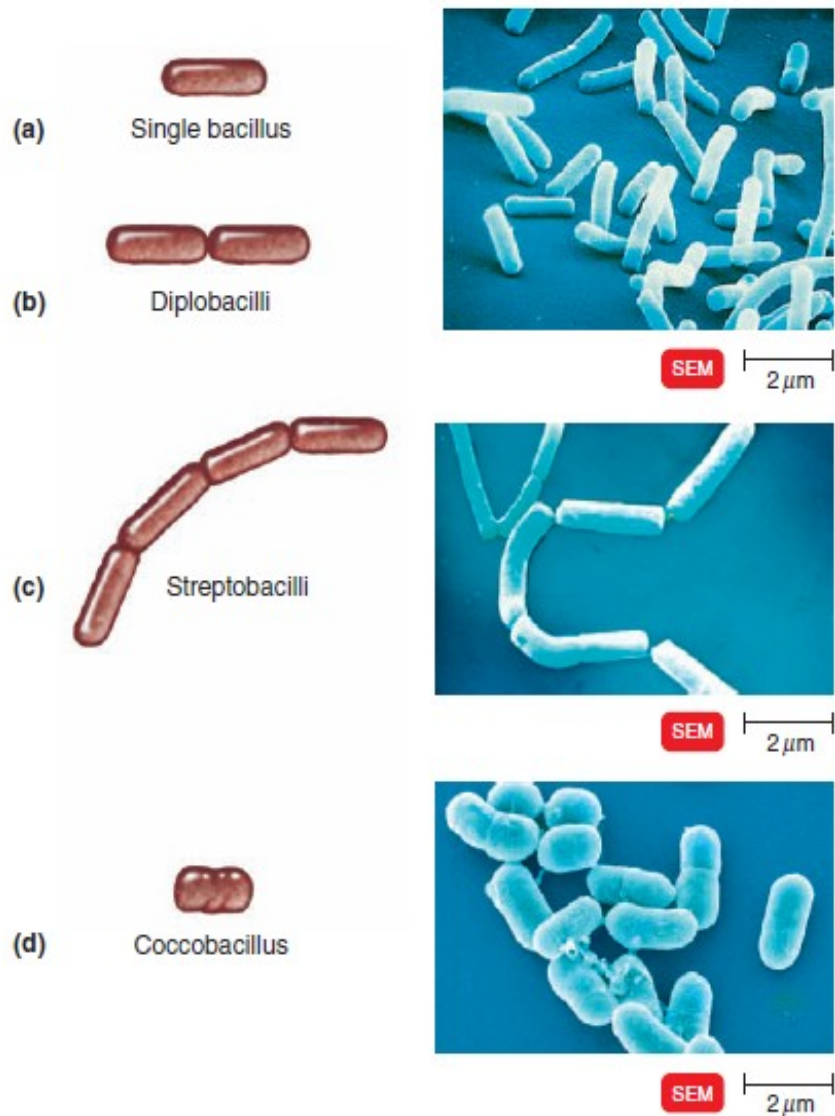


Figure 4.2 Bacilli. (a) Single bacilli. (b) Diplobacilli. In the top micrograph, a few joined pairs of bacilli could serve as examples of diplobacilli. (c) Streptobacilli. (d) Coccobacilli.

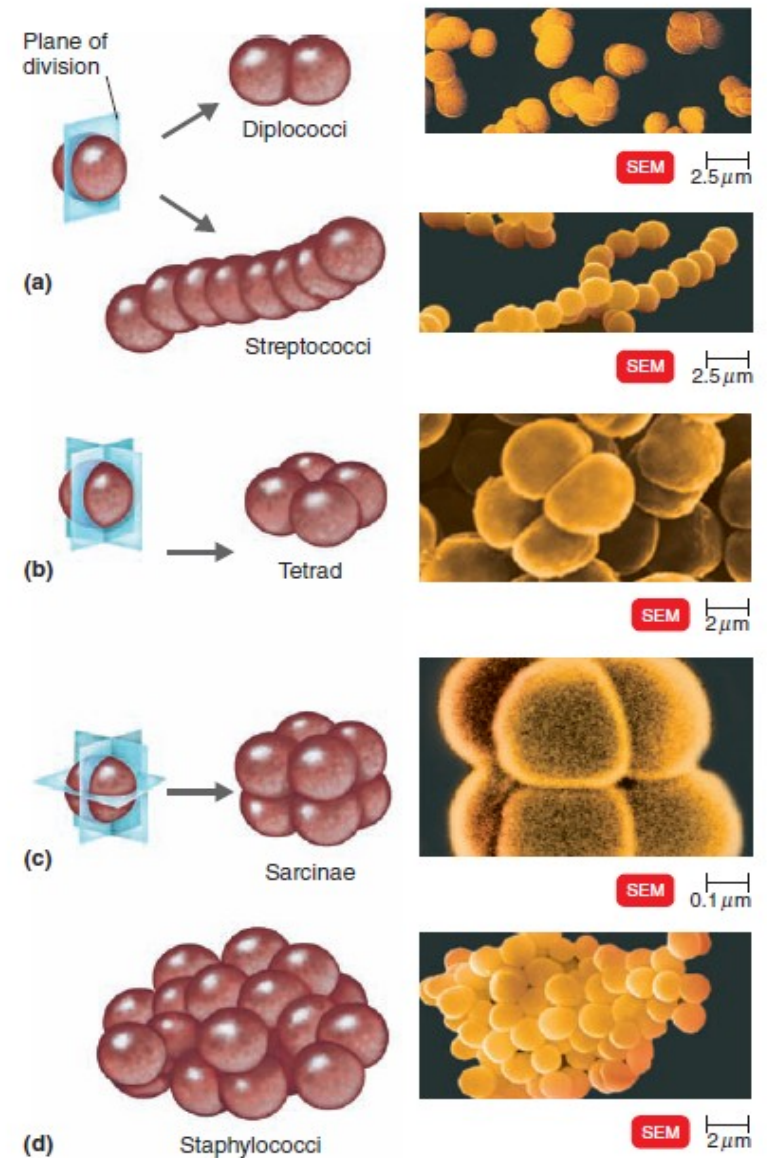
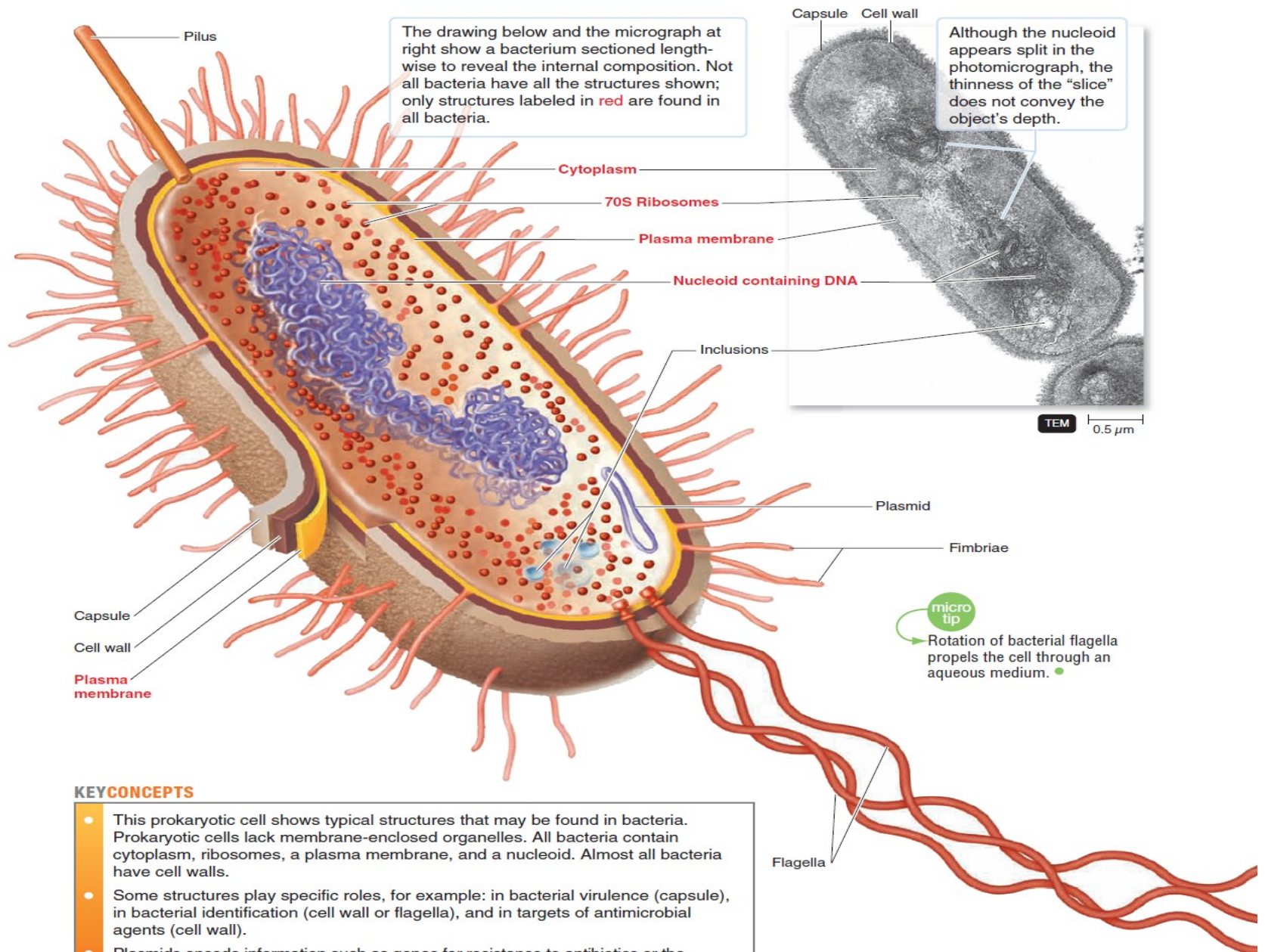


Figure 4.1 Arrangements of cocci. (a) Division in one plane produces diplococci and streptococci. (b) Division in two planes produces tetrads. (c) Division in three planes produces sarcinae, and (d) division in multiple planes produces staphylococci.



KEY CONCEPTS

- This prokaryotic cell shows typical structures that may be found in bacteria. Prokaryotic cells lack membrane-enclosed organelles. All bacteria contain cytoplasm, ribosomes, a plasma membrane, and a nucleoid. Almost all bacteria have cell walls.
- Some structures play specific roles, for example: in bacterial virulence (capsule), in bacterial identification (cell wall or flagella), and in targets of antimicrobial agents (cell wall).
- Plasmids encode information such as genes for resistance to antibiotics or the production of toxins. Plasmids may be exchanged between bacteria.

Cell wall:

- Chemically complex cell wall
- Protects bacteria against osmotic lysis
- Chemically composed of **Peptidoglycans** (also termed as **Murein**)
- In **Gram-Positive bacteria:** Peptidoglycans consists of a single 20 to 80 nm thick homogeneous layer lying outside the plasma membrane
- They additionally have acidic substances called teichoic acids in their cell wall
- **Teichoic acid** are polyolphosphate polymers (a polymer of glycerol or ribitol joined by phosphate) bearing a strong negative charge
- They can be covalently linked to N-acetylmuramic acid of the Peptidoglycan layer or to the lipids of the plasma membrane
- Main function of teichoic acid is to provide rigidity to the cell wall by

- In **Gram-Negative bacteria:** Cell wall consists of 2 to 7 nm thick Peptidoglycan layer covered by a 7 to 8 nm thick outer membrane
- **Mycoplasma**, which are the smallest known bacteria and are derived from eubacteria and they lack cell walls or have very little wall material
- L- form bacteria (**Bacillus**

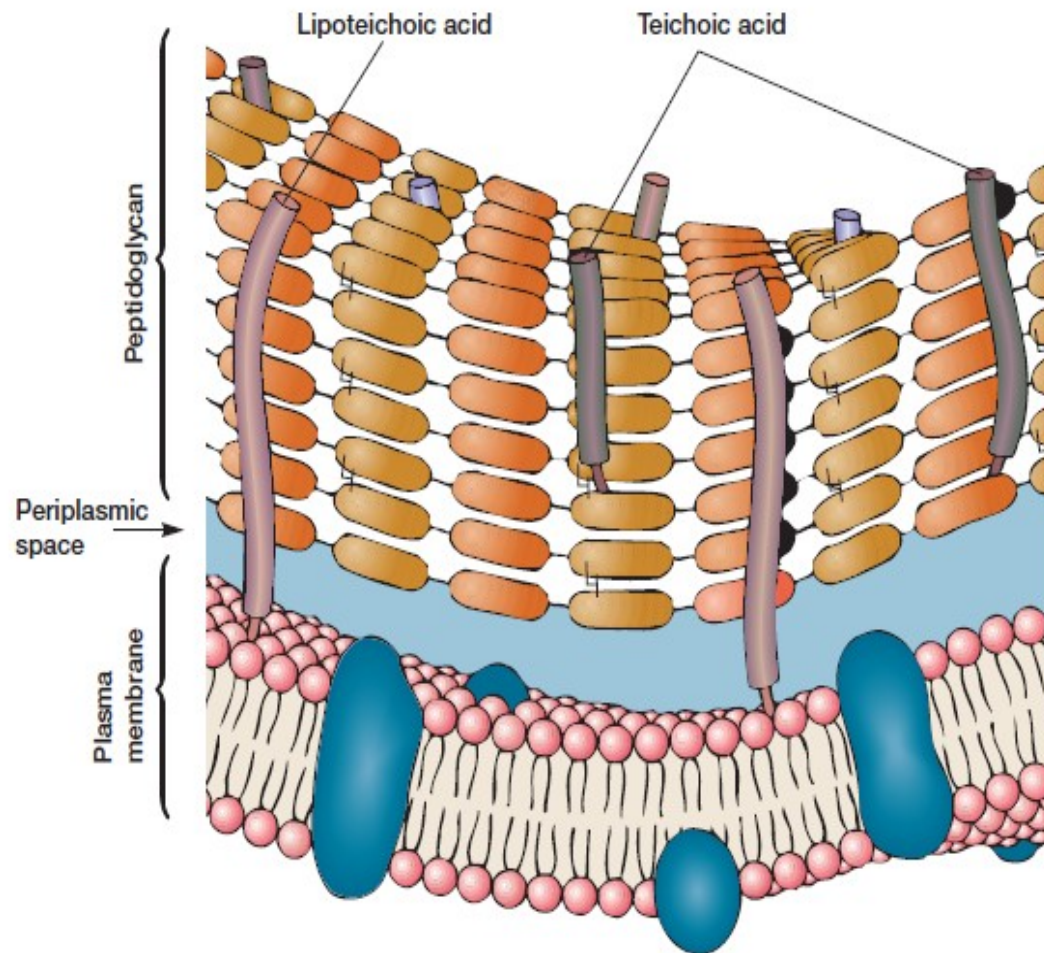


Figure 3.21 The Gram-Positive Envelope.

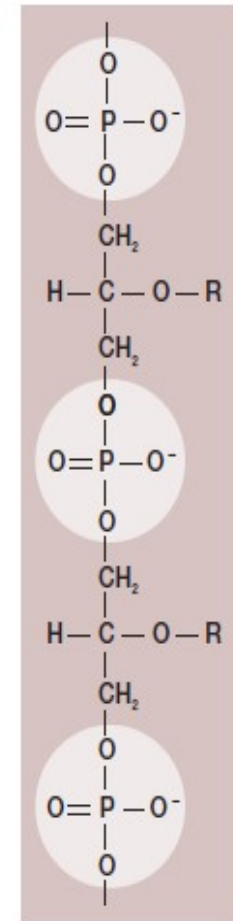


Figure 3.22 Teichoic Acid Structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent D-alanine, glucose, or other molecules.

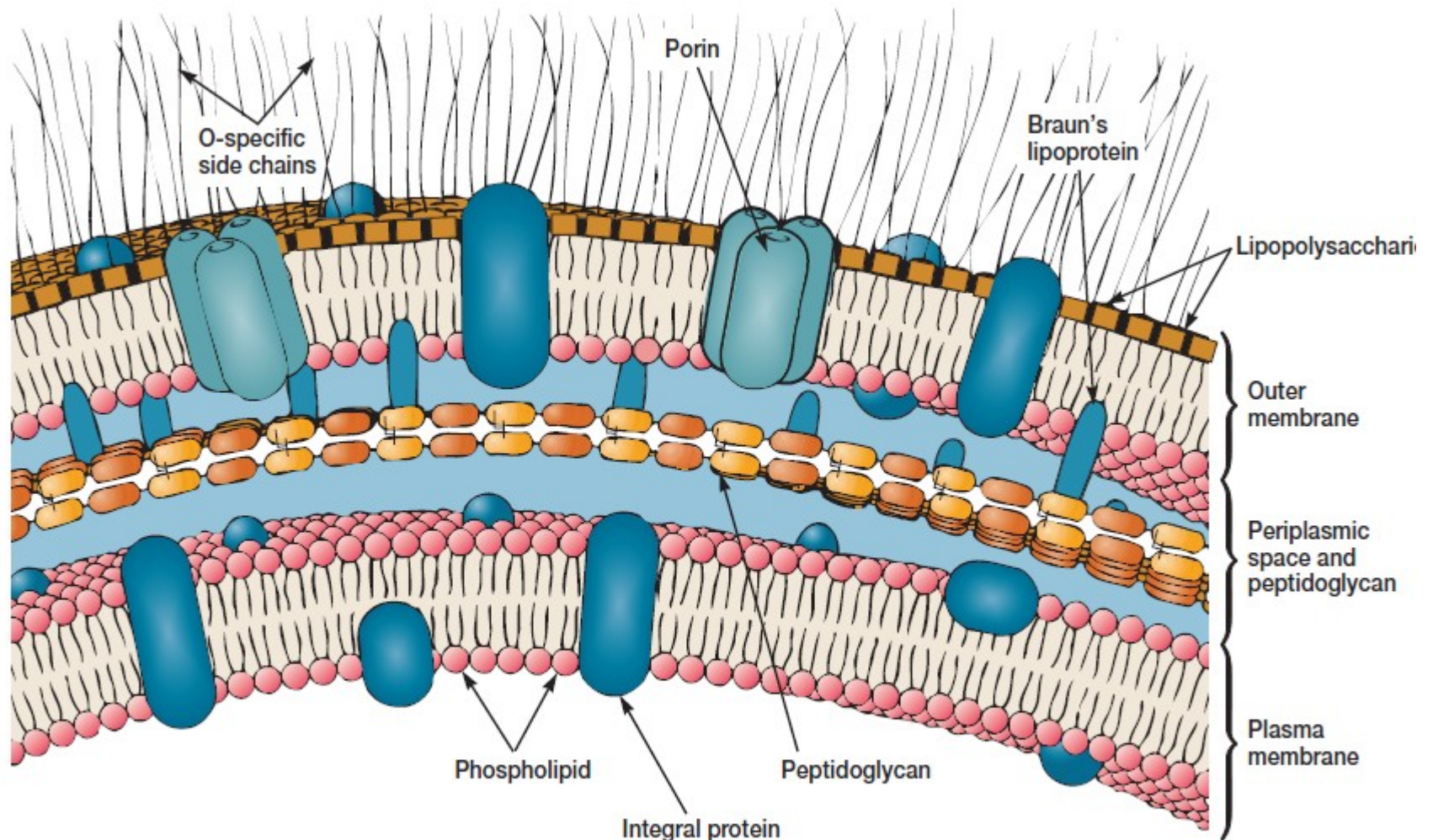
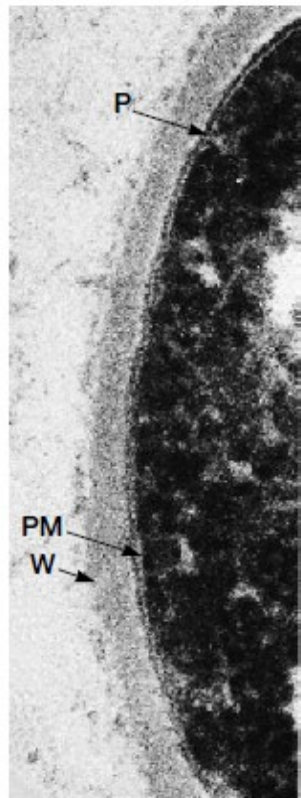
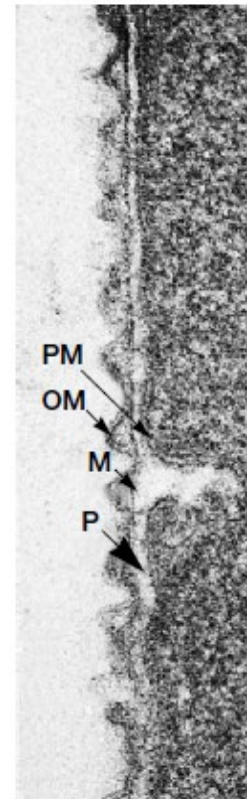
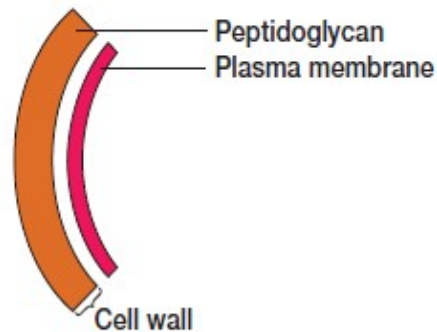


Figure 3.23 The Gram-Negative Envelope.



The gram-positive cell wall



The gram-negative cell wall

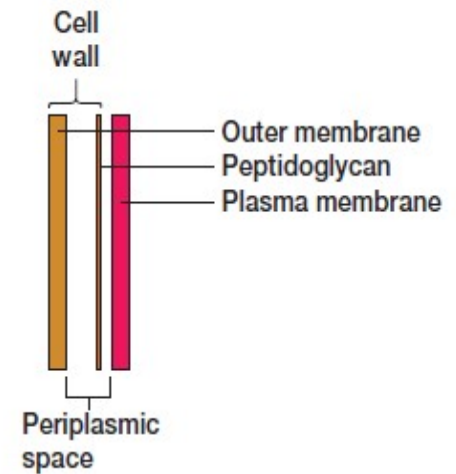


Figure 3.15 Gram-Positive and Gram-Negative Cell Walls. The gram-positive envelope is from *Bacillus licheniformis* (left), and the gram-negative micrograph is of *Aquaspirillum serpens* (right). M; peptidoglycan or murein layer; OM, outer membrane; PM, plasma membrane; P, periplasmic space; W, gram-positive peptidoglycan wall.

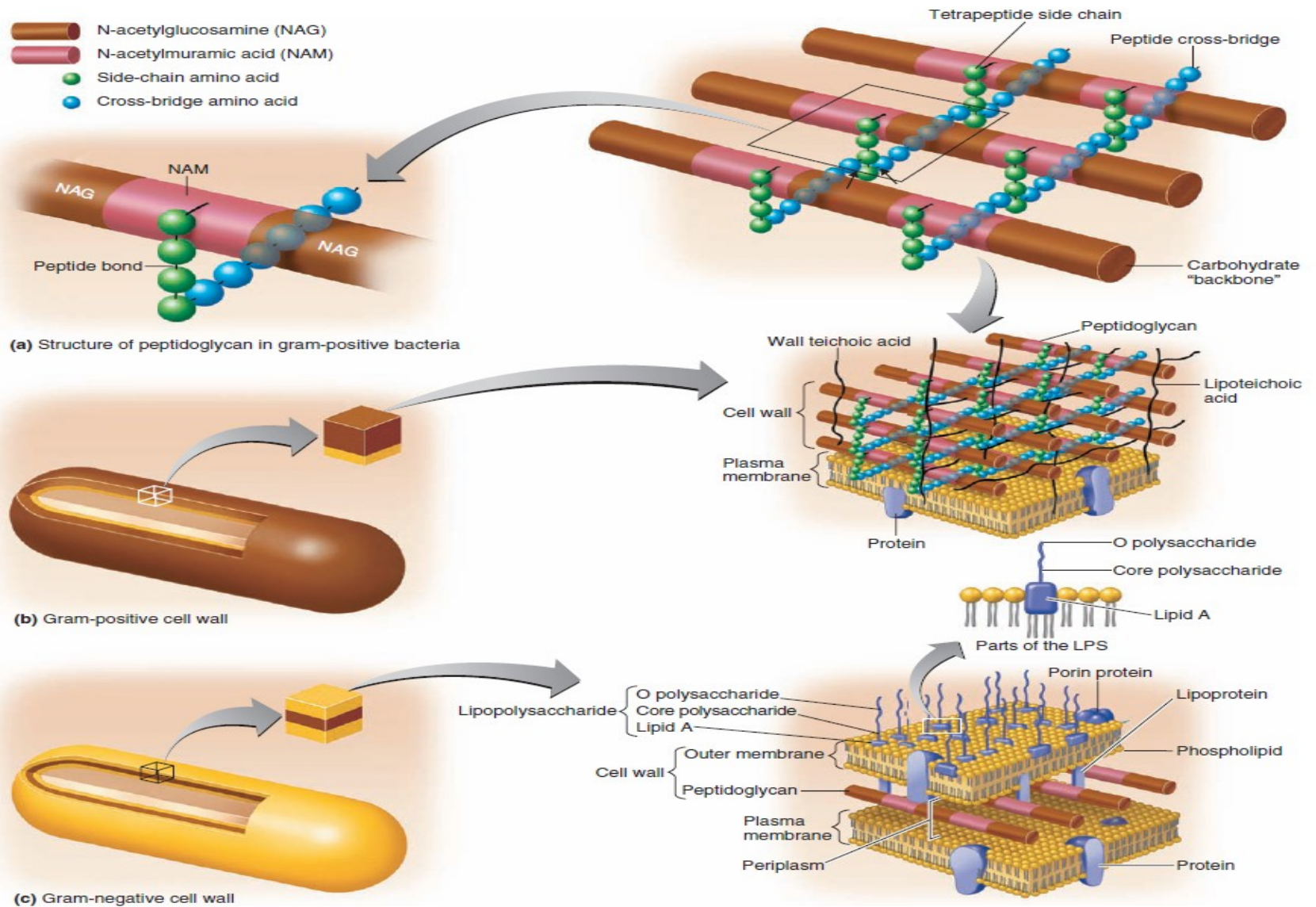


Figure 4.13 Bacterial cell walls. (a) The structure of peptidoglycan in gram-positive bacteria. Together the carbohydrate backbone (glycan portion) and tetrapeptide side chains (peptide portion) make up peptidoglycan.

The frequency of peptide cross-bridges and the number of amino acids in these bridges vary with species of bacteria. The small arrows indicate where penicillin interferes with the linkage of peptidoglycan rows by peptide

cross-bridges. (b) A gram-positive cell wall. (c) A gram-negative cell wall.

Q What are the major structural differences between gram-positive and gram-negative cell walls?

Peptidoglycan is a polymer containing two sugar derivatives **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** joined through **β -1,4 glycosidic bond**. **NAM** is **NAG** with **lactic acid** attached by **ether linkage**.

A peptide chain of four alternating **D- and L- amino acids** called **tetrapeptide** is connected to the carboxyl group of the **NAM**.

Amino acids present in tetrapeptide are- **L-alanine, D-alanine, D-**

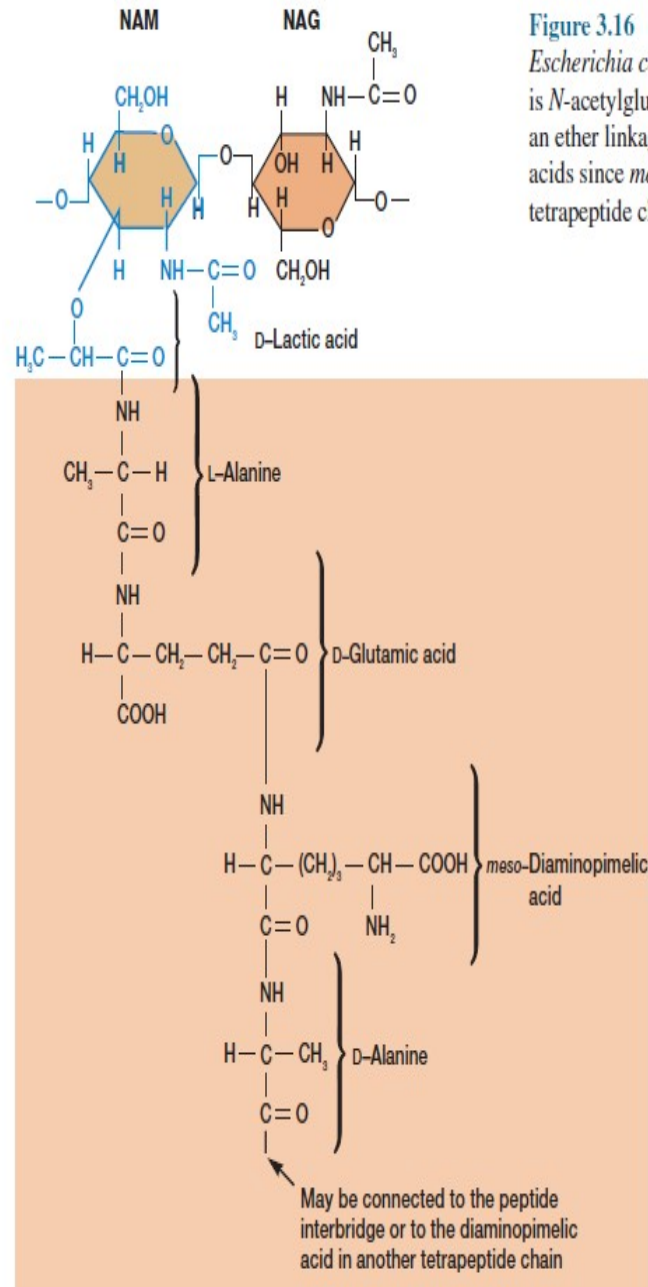


Figure 3.16 Peptidoglycan Subunit Composition. The peptidoglycan subunit of *Escherichia coli*, most other gram-negative bacteria, and many gram-positive bacteria. NAG is *N*-acetylglucosamine. NAM is *N*-acetylmuramic acid (NAG with lactic acid attached by an ether linkage). The tetrapeptide side chain is composed of alternating D- and L-amino acids since *meso*-diaminopimelic acid is connected through its L-carbon. NAM and the tetrapeptide chain attached to it are shown in different shades of color for clarity.

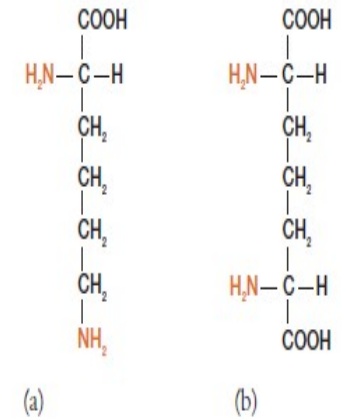
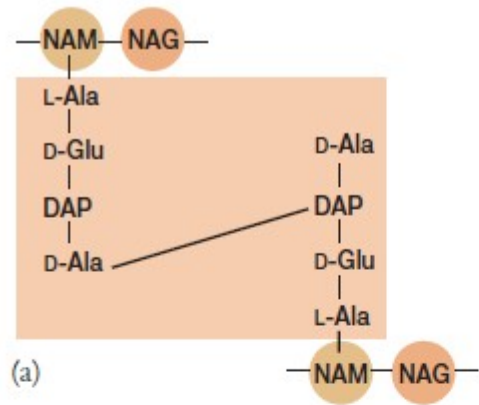


Figure 3.17 Diaminoacids Present in Peptidoglycan. (a) L-Lysine. (b) *meso*-Diaminopimelic acid.



2 tetrapeptide chains lying side-by-side may be linked together directly to each other or indirectly by a peptide interbridge, consisting of a short chain amino acids

The reaction that forms the peptide cross-links during Peptidoglycan synthesis is called **Transpeptidation**

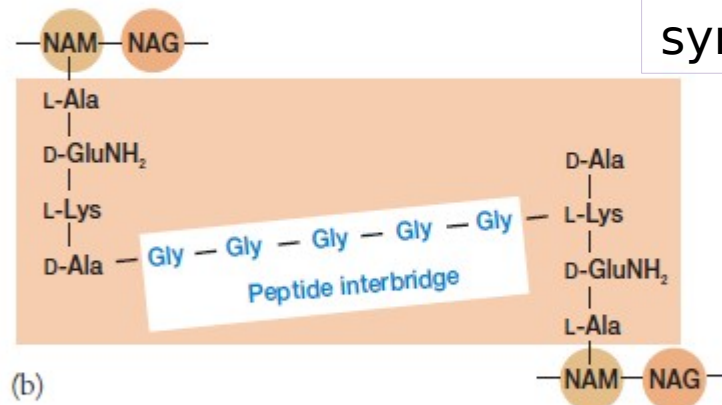


Figure 3.18 Peptidoglycan Cross-Links. (a) *E. coli* peptidoglycan with direct cross-linking, typical of many gram-negative bacteria. (b) *Staphylococcus aureus* peptidoglycan. *S. aureus* is a gram-positive bacterium. NAM is *N*-acetylmuramic acid. NAG is *N*-acetylglucosamine. Gly is glycine. Although the polysaccharide chains are drawn opposite each other for the sake of clarity, two chains lying side-by-side may be linked together (see figure 3.19).

Outer membrane

- In addition to Peptidoglycan cell wall, the Gram-Negative bacterium contain an additional membrane, **The Outer Membrane**
- The constituents of this layer are not completely similar to the plasma membrane
- It contains **Lipopolysaccharides, Lipoproteins, Proteins** and **Phospholipids**
- **Lipid + Polysaccharides** linked in outer membrane = **Lipopolysaccharide**
Because of this, the outer membrane is often called the **Lipopolysaccharide Layer (LPS Layer)**
- The LPS consists of 3 components,
 1. The **Core Polysaccharide**
 2. The **O-polysaccharide** (also called **O-side chain** or **O-antigen**)- functions as an antigen and is useful for distinguishing species of Gram- Negative bacteria
 3. **Lipid Portion-** lipid A (contains 2 glucosamine sugar derivatives, each with fatty acids and phosphate or pyrophosphate attached)
 - Lipid A often is toxic, as a result the LPS can act as an **Endotoxin**
- Outer membrane of Gram- Negative bacteria is relatively more permeable as compared to the plasma membrane due to the

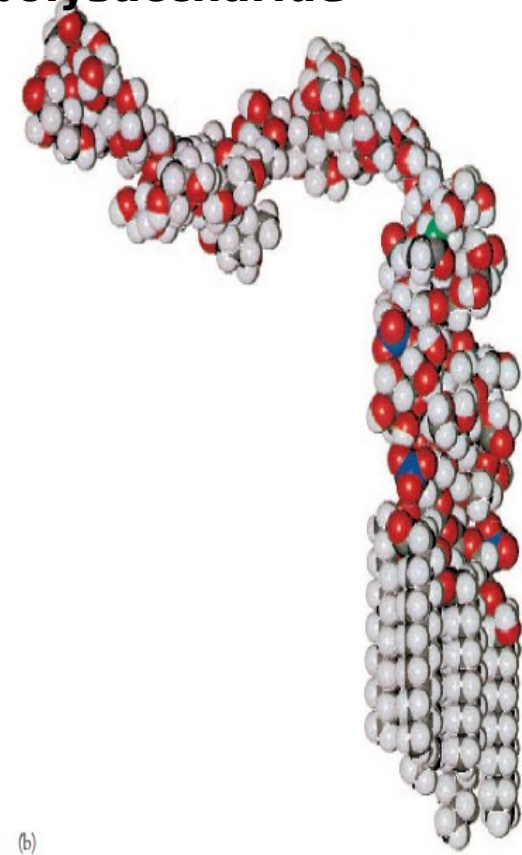
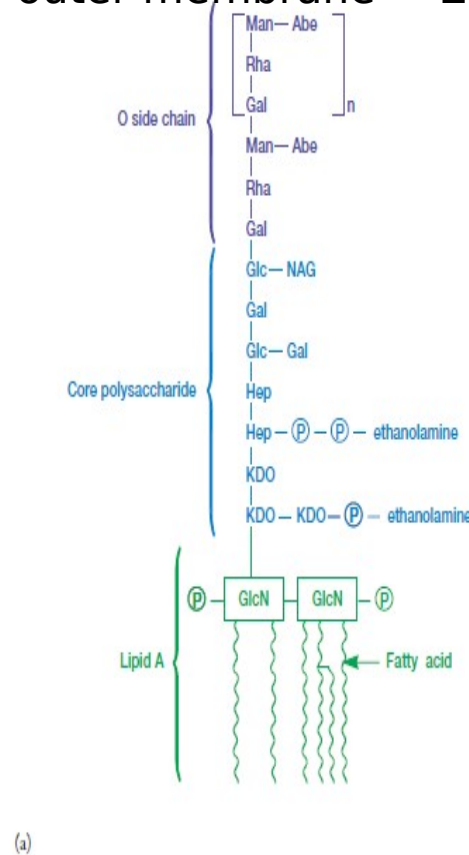


Figure 3.25 Lipopolysaccharide Structure. (a) The lipopolysaccharide from *Salmonella*. This slightly simplified diagram illustrates one form of the LPS. Abbreviations: Abe, abequeose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Hep, heptulose; KDO, 2-keto-3-deoxyoctonate; Man, mannose; NAG, *N*-acetylglucosamine; P, phosphate; Rha, L-rhamnose. Lipid A is buried in the outer membrane. (b) Molecular model of an *Escherichia coli* lipopolysaccharide. The lipid A and core polysaccharide are straight; the O side chain is bent at an angle in this model.

Glycocalyx:

- Thick, high-molecular weight secretory substance
- Present in many bacteria external to the cell wall
- Composed of polysaccharide, polypeptide or both
- Glycocalyx may be thick or thin, rigid or flexible, depending on their chemical nature

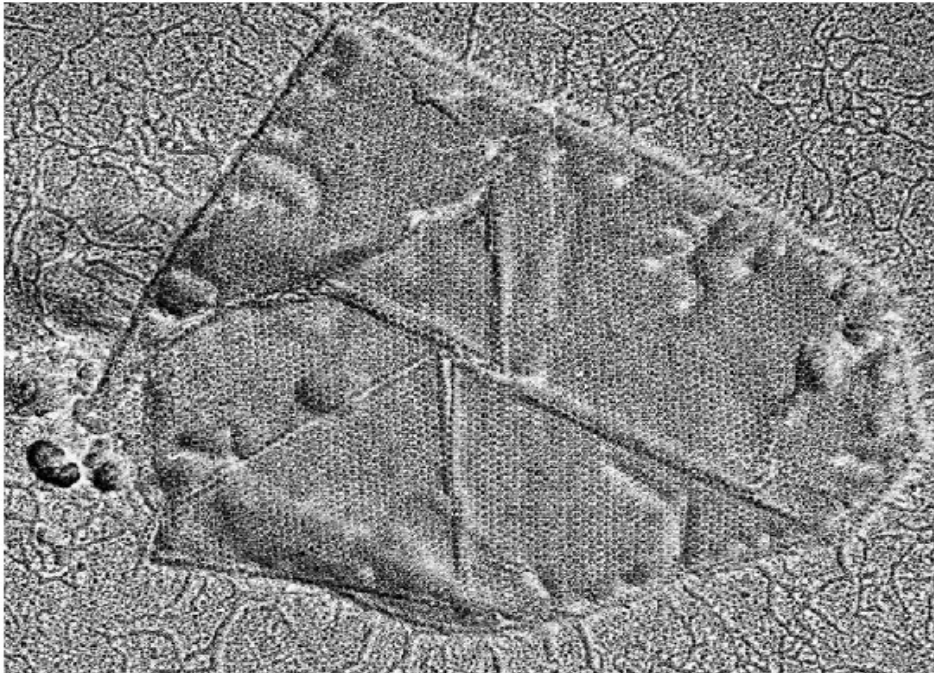


Figure 3.29 The S-Layer. An electron micrograph of the S-layer of *Deinococcus radiodurans* after shadowing.

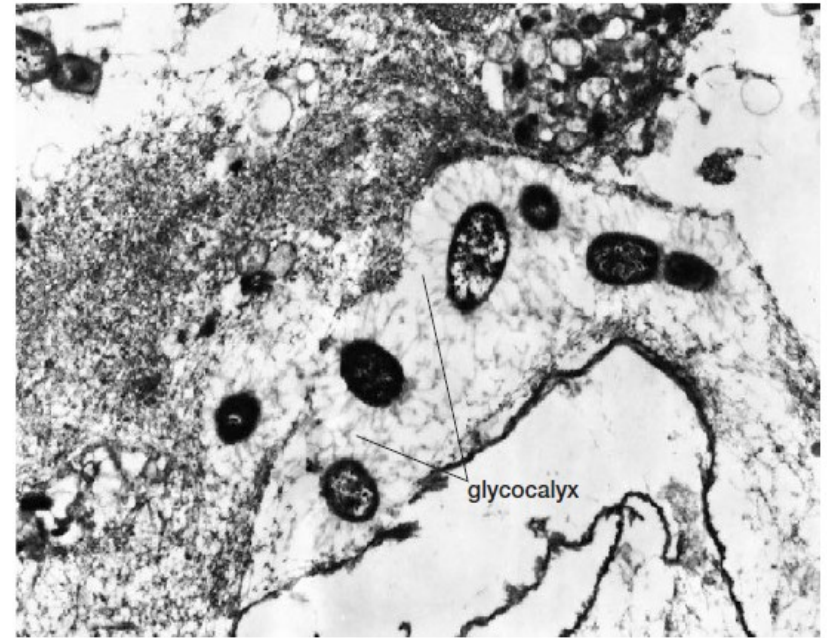


Figure 3.28 Bacterial Glycocalyx. Bacteria connected to each other and to the intestinal wall, by their glycocalyxes, the extensive networks of fibers extending from the cells ($\times 17,500$).

- If the layer is more easily deformed and loosely attached to the cell wall= **Slime Layer**
- Most capsules consist of polysaccharides; some bacilli (***Bacillus anthracis***) consists of polypeptides, mainly poly-D-glutamic acid
- The possession of capsules makes

Plasma membrane:

- Unit membrane composed primarily of protein and phospholipid
- Structurally and chemically similar to eukaryotic plasma membrane (major difference is eukaryotes have **sterols**; prokaryotes have **hopanoids** (similar to sterols))
- **Functions of plasma membrane:** Transport, Energy Transduction, Invagination of the plasma membrane in the shape of vesicles, tubules or lamellae (called **Mesosome**) may be involved in chromosome replication or cross wall formation in dividing bacteria
- **Mesosomes** are present in both Gram-Positive and Gram-Negative bacteria

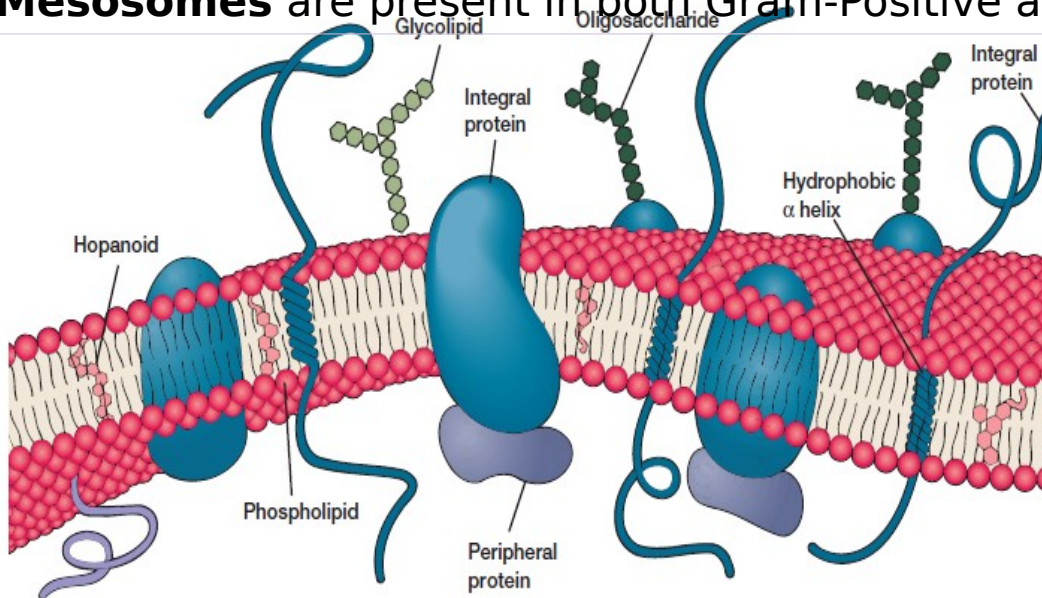


Figure 3.7 Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (pink) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

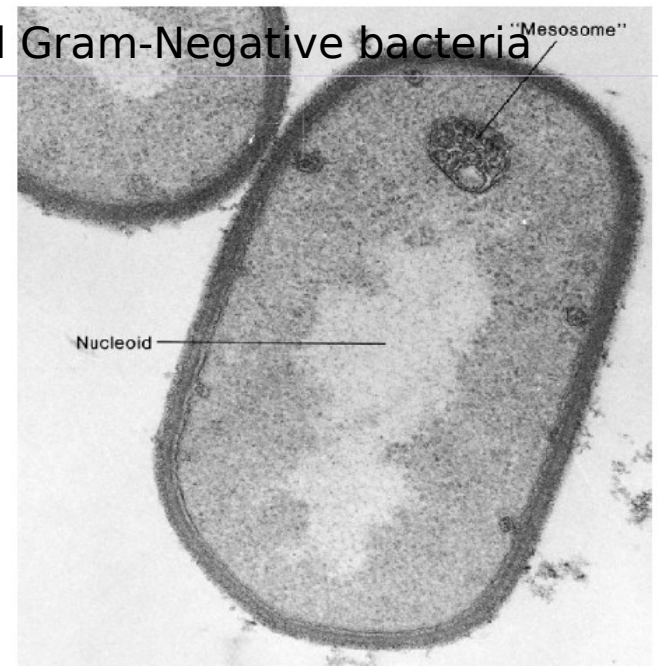


Figure 3.8 Mesosome Structure. *Bacillus fastidiosus* ($\times 91,000$). A large mesosome lies adjacent to the nucleoid.

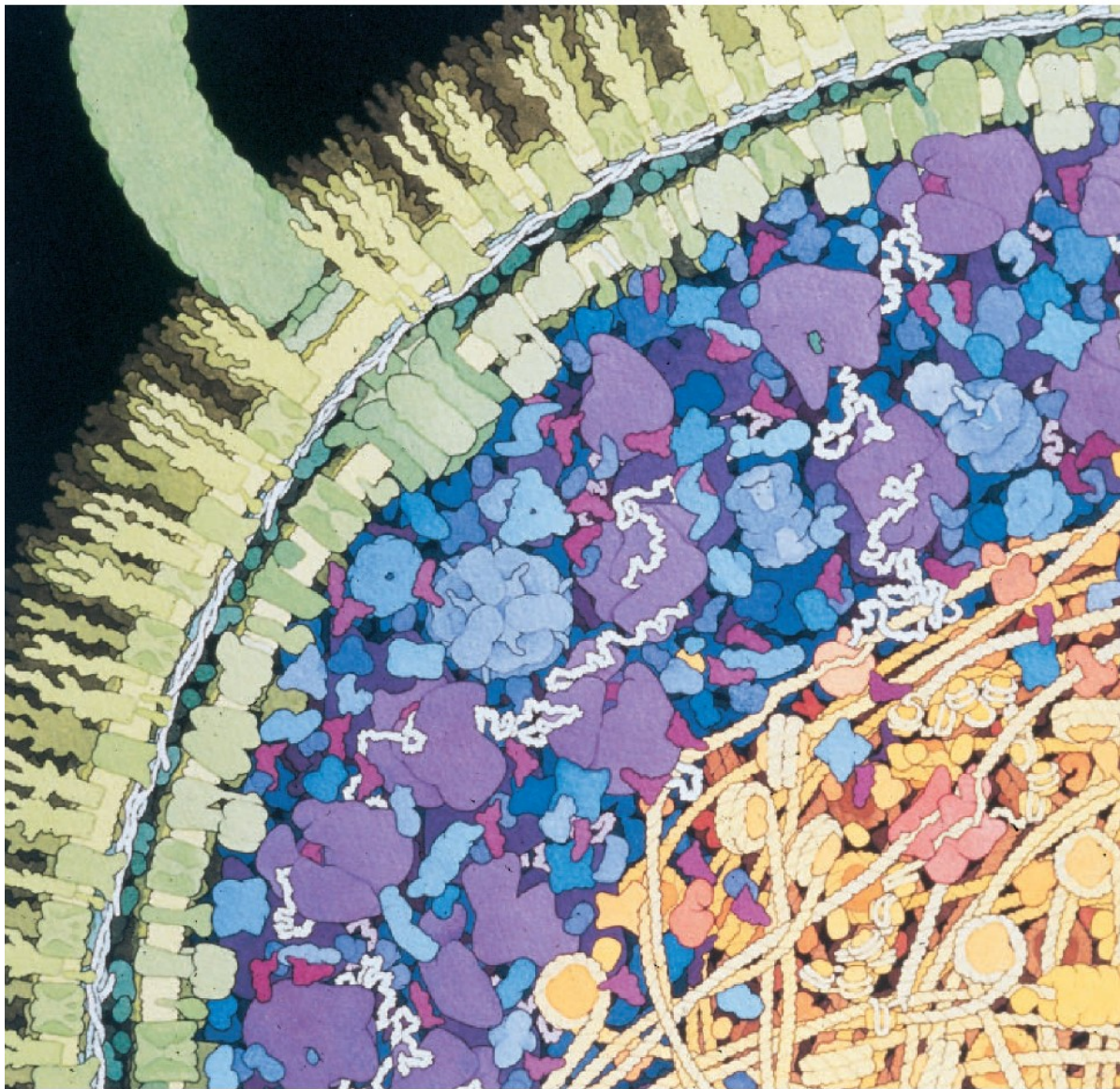
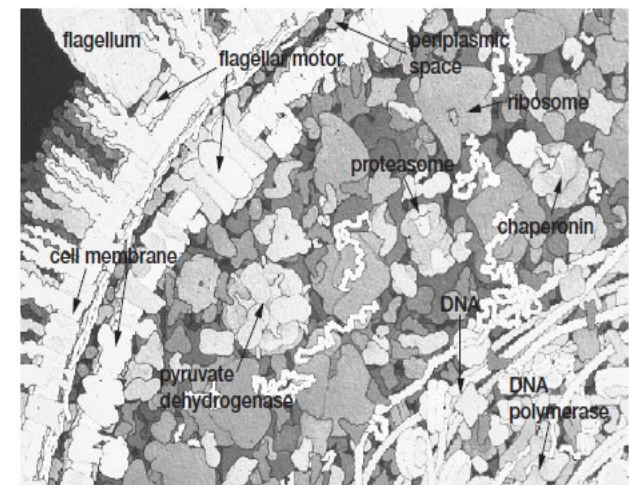


Figure 3.10 A Cross Section of the Bacterium *Escherichia coli* Drawn at a Magnification of a Million Times. The glycocalyx, flagellum, gram-negative cell wall, and plasma membrane are at the top. Ribosomes synthesizing proteins fill the underlying cytoplasmic matrix. At the bottom is the nucleoid with its dense tangle of DNA and associated proteins.



Cytoplasm:

- Refers to substance of the cell inside the plasma membrane
- Cytoplasm is about 80% water and contains primarily proteins, carbohydrates, lipids, inorganic ions and many low molecular weight compounds
- Lacks a unit membrane bound organelles
- Major **Structure Present In The Cytoplasm Are Ribosomes And Reserve Deposits Called** inclusion bodies

1. Ribosome- 70S type- subunits of a 70S ribosome are a small 30S subunit containing one molecule of rRNA (16S rRNA) and a large 50S subunit containing two molecules of rRNA (23S and 5S rRNA)

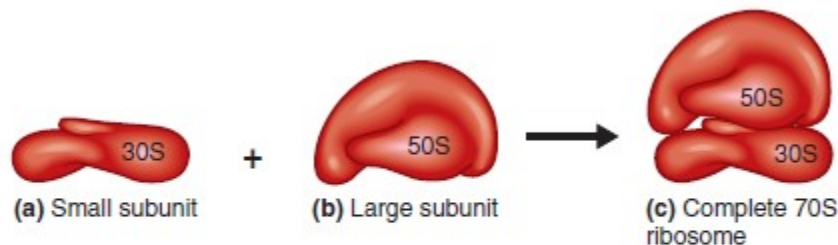
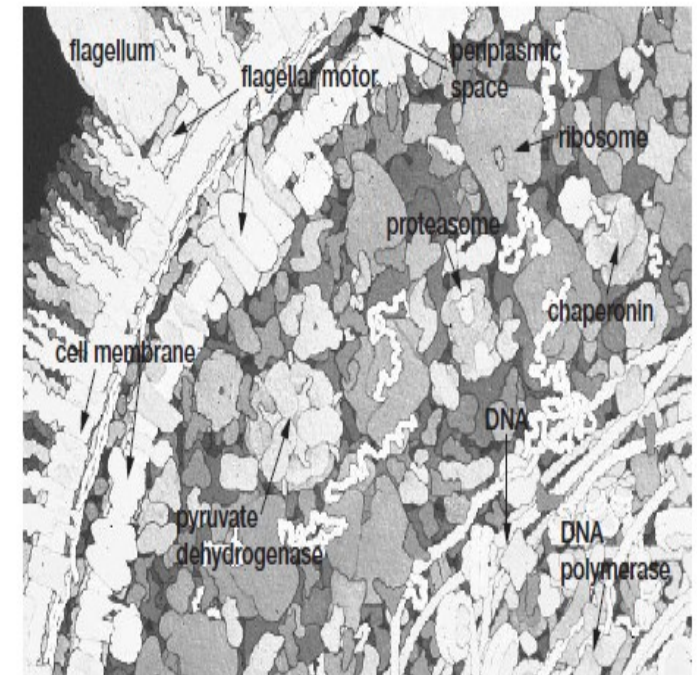


Figure 4.19 The prokaryotic ribosome. (a) A small 30S subunit and (b) a large 50S subunit make up (c) the complete 70S prokaryotic ribosome.

Figure 3.10 A Cross Section of the Bacterium *Escherichia coli* Drawn at a Magnification of a Million Times. The glycocalyx, flagellum, gram-negative cell wall, and plasma membrane are at the top. Ribosomes synthesizing proteins fill the underlying cytoplasmic matrix. At the bottom is the nucleoid with its dense tangle of DNA and associated proteins.



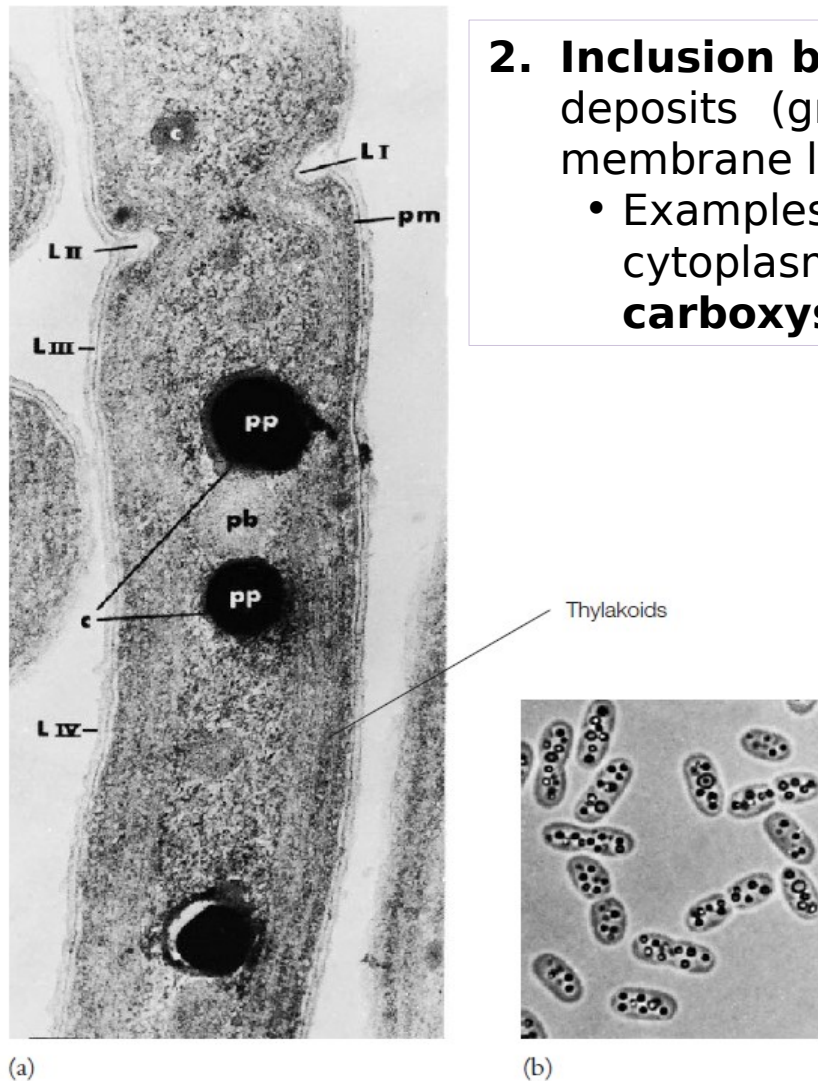


Figure 3.13 Inclusion Bodies in Bacteria. (a) Ultrastructure of the cyanobacterium *Anacystis nidulans*. The bacterium is dividing, and a septum is partially formed, LI and LII. Several structural features can be seen, including cell wall layers, LIII and LIV; the plasma membrane, pm; polyphosphate granules, pp; a polyhedral body, pb; and cyanophycin material, c. Thylakoids run along the length of the cell. Bar = 0.1 μm. (b) *Chromatium vinosum*, a purple sulfur bacterium, with intracellular sulfur granules, light field (×2,000).

2. Inclusion bodies-

Contains organic and inorganic reserve deposits (granules) that may be membrane bound or membrane less

- Examples of inclusion bodies present in bacterial cytoplasm are **glycogen, cyanophycin granules, carboxysomes, polyphosphate granules** and others.

Glycogen- It is the polymer of glucose. Present in the form of granules in the cytosol. In the presence of iodine= granules appear reddish brown

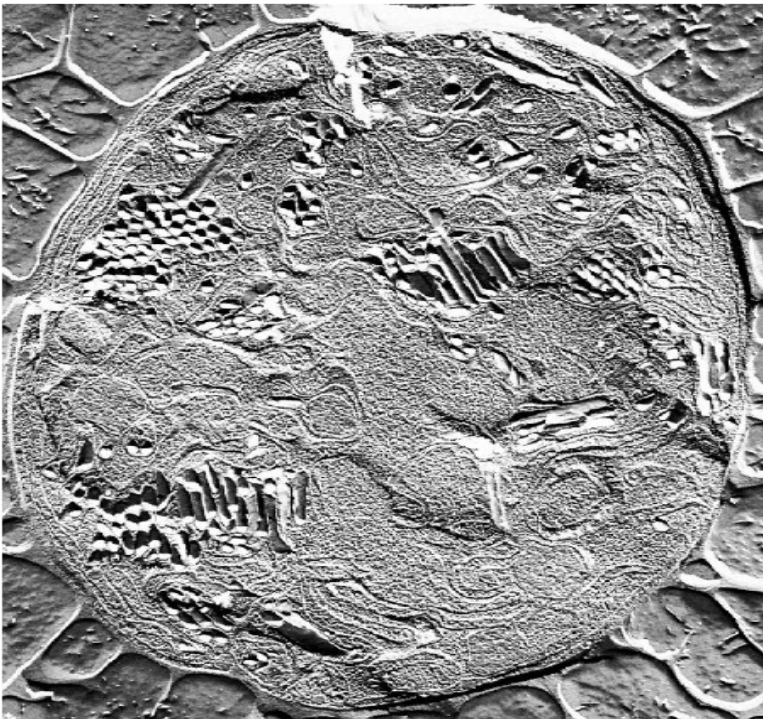
- **Cyanophycin granules**- Composed of large polypeptides containing approximately equal amounts of amino acids, Arginine and Aspartic acid. It is non- ribosomally produced amino acid polymer and present in cyanobacteria and few heterotrophic bacteria
- **Carboxysomes**- Polyhedral inclusion bodies that contain the CO₂ fixation enzymes-ribulose-1,5-bisphosphate carboxylase (RuBisCO)
 - These structures are found in all cyanobacteria and in many chemotrophic bacteria that fixes carbon dioxide

- **Polyphosphate granules (Volutin granules)**- Reserve form of inorganic phosphate (polyphosphate) present in the cytoplasm of some bacteria

- Volutin granules show red or a different shade of blue when stained with blue basic

- **Magnetosomes-** Membrane bound structure consisting of magnetic mineral crystal [crystals of magnetite (Fe_3O_4) / crystals of greigite (Fe_3S_4)] surrounded by a lipid bilayer and present in magnetotactic bacteria

- It acts like compass needle to direct in



(b)

Figure 3.12 Gas Vesicles and Vacuoles. (a) Filaments of the cyanobacterium *Anabaena flos-aquae* as seen in the light microscope. (b) A freeze-fracture preparation of *Anabaena flos-aquae* ($\times 89,000$). Clusters of the cigar shaped vesicles form gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles can be seen.

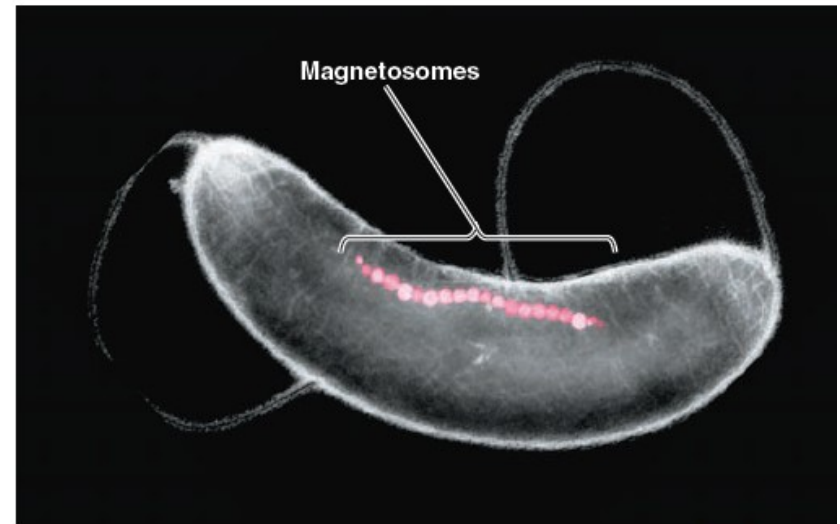
TEM 1 μm

Figure 4.20 Magnetosomes. This micrograph of *Magnetospirillum magnetotacticum* shows a chain of magnetosomes. This bacterium is usually found in shallow freshwater mud.

3. Gas vesicle- Hollow spindle-shaped structure made of protein

- It is impermeable to liquid water, but it is highly permeable to gases and is normally filled with air
- It is a rigid structure of low compressibility
- It provides buoyancy to planktonic cells by decreasing their overall cell density

Surface appendages:

3 types of appendages recognized in bacterial species

- **Flagella**- Organs of **Locomotion**
- **Pili**- Meant for **Conjugation**
- **Fimbriae**- Meant for **Attachment**

Flagella:

- Occur in both Gram-Positive and Gram-Negative bacteria
- **Structure**- long, filamentous surface appendages
- Flagellum of Gram-Negative bacteria (*E. coli*) consists of 3 parts:
 1. **Long filament**- which lies external to the cell surface
 2. **Hook structure**- at the end of the filament
 3. **Basal body**- to which the hook is anchored and which imparts motion to the flagellum

Pili:

- Singular *pilus*
- Thin, hair like rigid appendages on the surface of bacteria
- Occur extensively in Gram-Negative bacteria
- Only few Gram-Positive (*Corynebacterium renale*) bear pili
- Proteins that form pili referred to as **pilins**
- **Function:**
 - Important role in the process of conjugation (hence sometimes called sex pili)

Fimbriae:

- Functions as receptor for donor-specific (male specific) phages
- Filamentous cell appendages
- Present in both Gram-Positive and Gram-Negative bacteria
- Composed of helically arranged protein subunits
- They mediate cell attachment to surface
- It can occur at the poles of the bacterial cell or can be evenly distributed over the entire surface of the cell

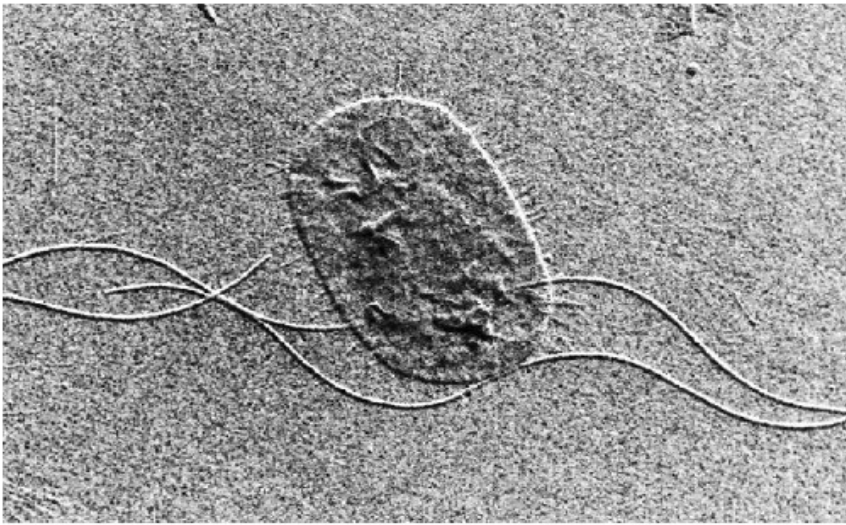


Figure 3.30 Flagella and Fimbriae. The long flagella and the numerous shorter fimbriae are very evident in this electron micrograph of *Proteus vulgaris* ($\times 39,000$).

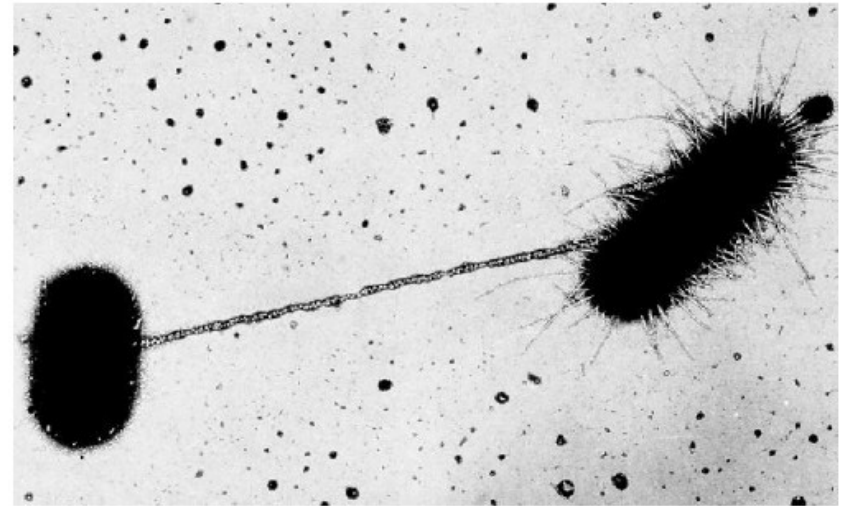


Figure 13.6 Bacterial Conjugation. An electron micrograph of two *E. coli* cells in an early stage of conjugation. The F^+ cell to the right is covered with small pili or fimbriae, and a sex pilus connects the two cells.



Figure 4.11 Fimbriae. The fimbriae seem to bristle from this *E. coli* cell, which is beginning to divide.

1. Flagellar filament:

- It is made up of **flagellin** protein
- Flagellin proteins are assembled to form a cylindrical structure with a hollow core
- The filament is rigid

2. Hook: Flexible

3. Basal body:

- It comprises of 4 rings (**M**, **S**, **P** and **L** rings)
- M- for **Membrane**= **Membrane Ring**; S for **Supramembranous** = **Supramembranous Ring**; P for **Peptidoglycan**= **Peptidoglycan Ring**; L for **Lipopolysaccharide-Lipopolysaccharide Ring**), later both M and S ring was merged together and called it as **MS-ring**
- Therefore they function as a unit
- 4th ring, **C-ring** is present inside the cell
- **C-ring** consists of three proteins, **FlhG**, **FlhM** and **FlhN**
- Both **MS-** and **C-ring** act as a Rotor

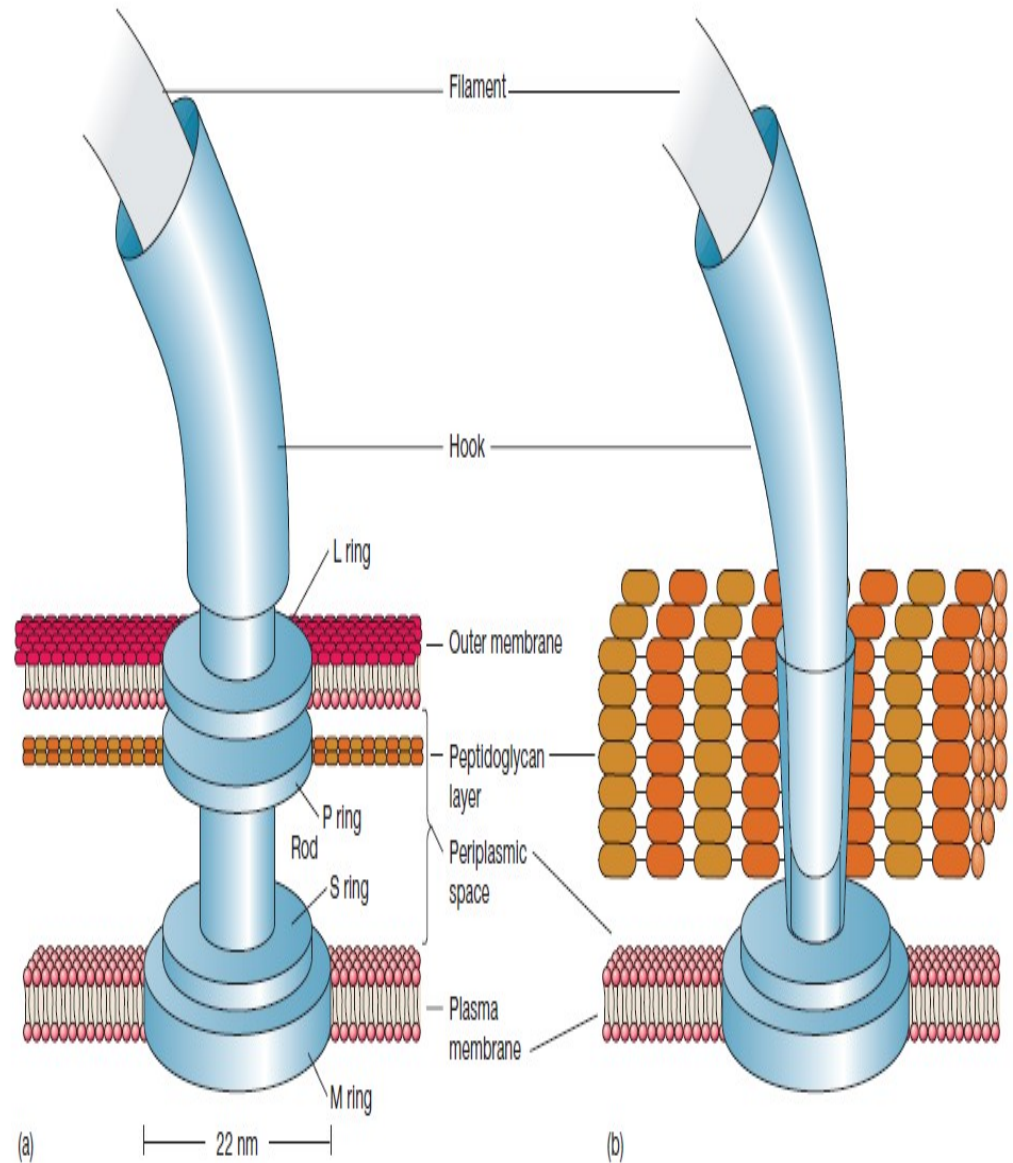
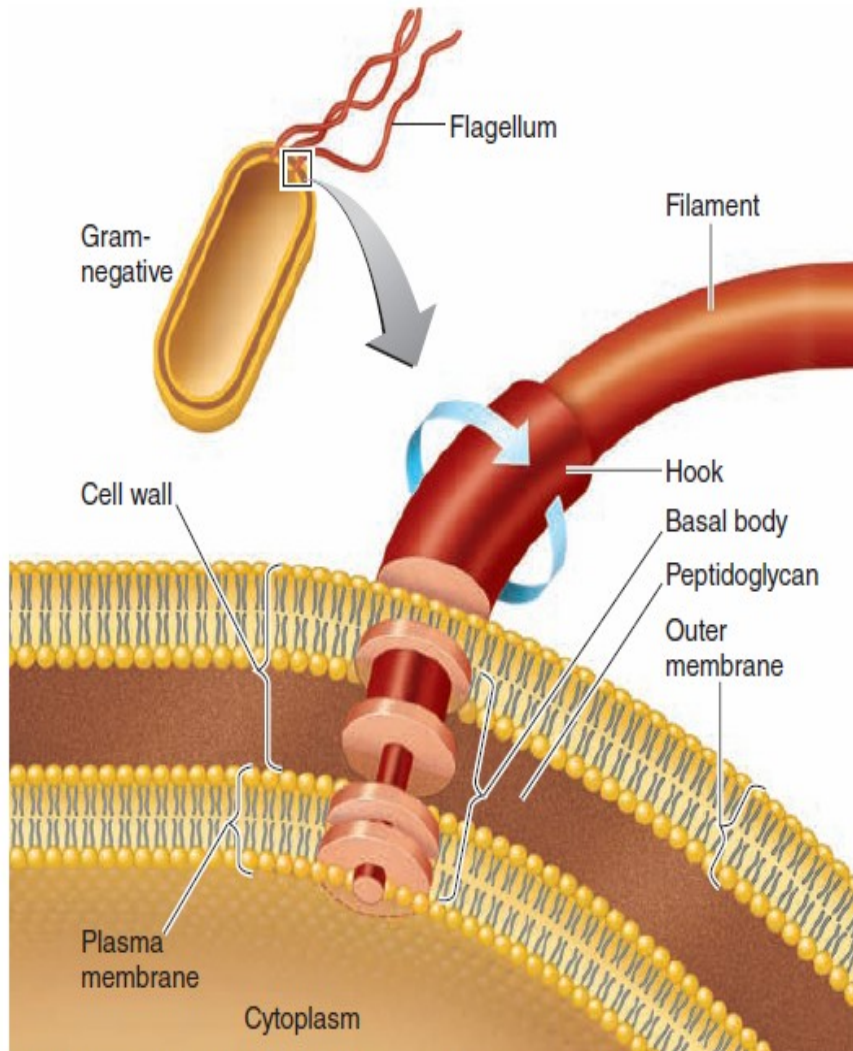
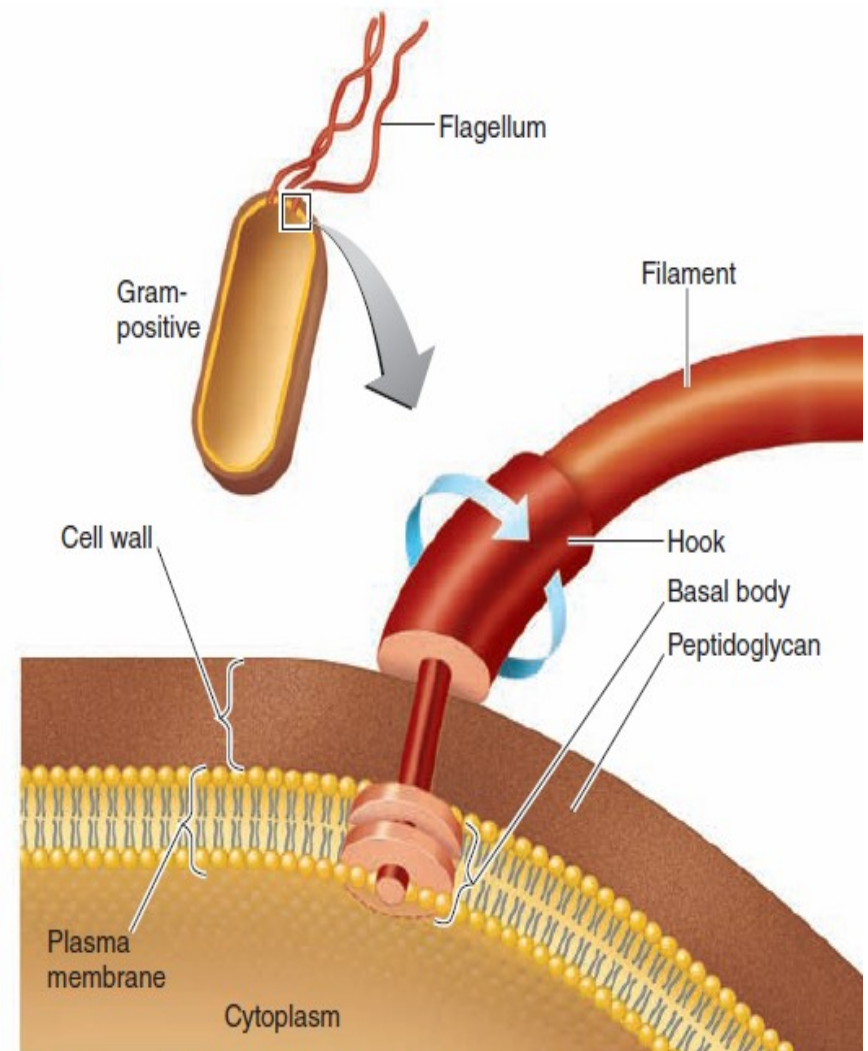


Figure 3.33 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) gram-negative and (b) gram-positive bacteria.



(a) Parts and attachment of a flagellum of a gram-negative bacterium



(b) Parts and attachment of a flagellum of a gram-positive bacterium

Figure 4.8 The structure of a prokaryotic flagellum. The parts and attachment of a flagellum of a gram-negative bacterium and gram-positive bacterium are shown in these highly schematic diagrams.

Based on number and patterns of distribution of flagella, bacteria are grouped as:

- **Monotrichous-** (**Trichous** means hair); having one flagellum
- **Amphitrichous-** (**Amphi** means on both sides); one flagellum attached at each end
- **Lophotrichous-** (**Lopho** meaning tuft); Cluster of flagella at one end or both ends
- **Peritrichous** - flagella are distributed all over the whole



(a) Peritrichous

SEM | 0.6 μ m



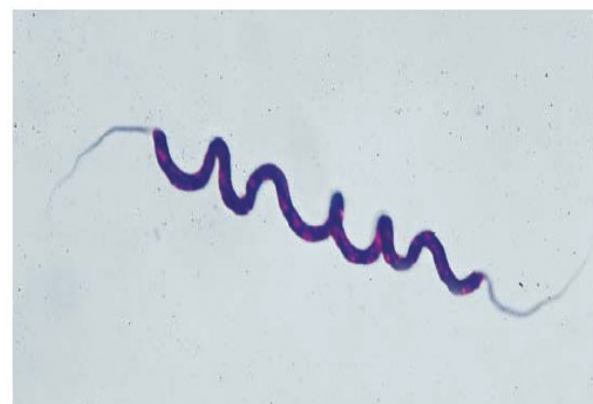
(b) Monotrichous and polar

SEM | 1 μ m



(c) Lophotrichous and polar

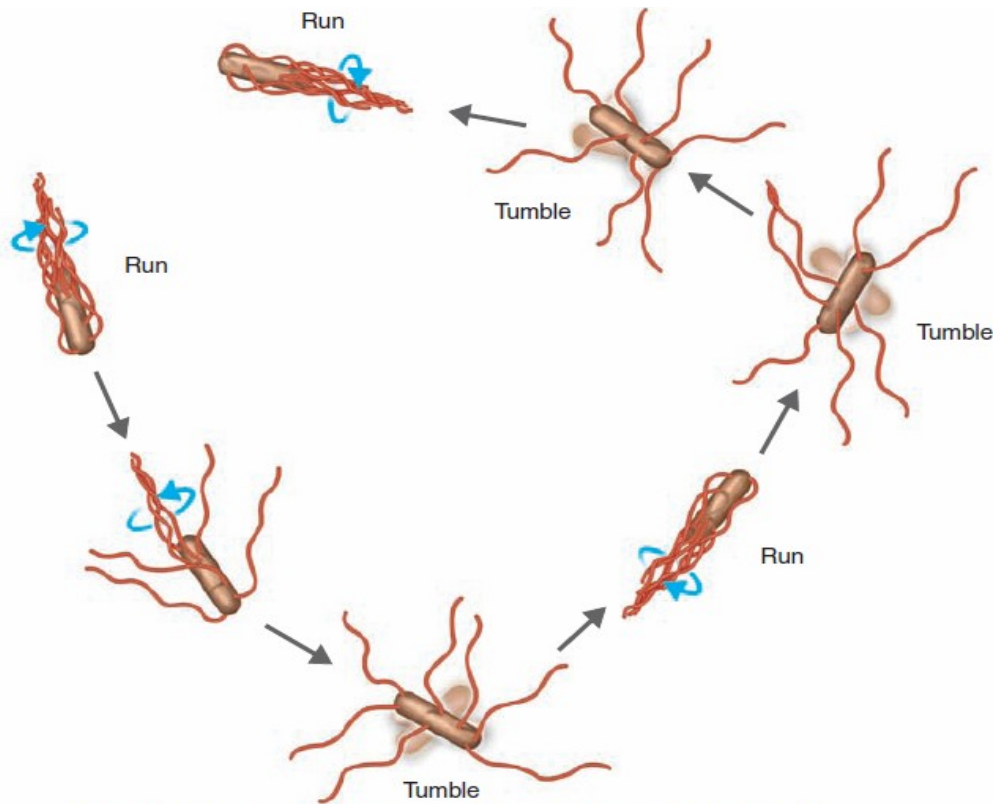
SEM | 1 μ m



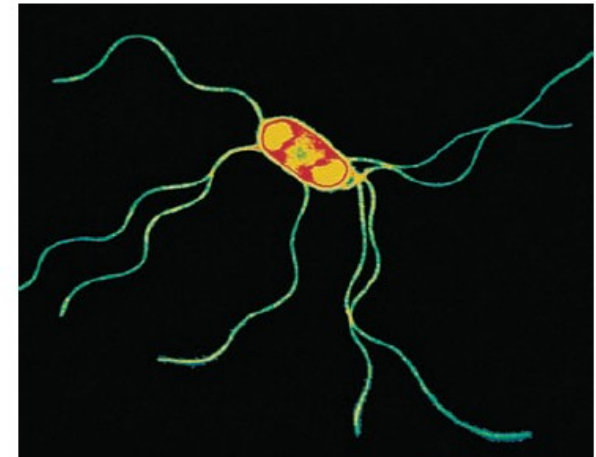
(d) Amphitrichous and polar

SEM | 10 μ m

Figure 4.7 Arrangements of bacterial flagella. (a) Peritrichous. (b)–(d) Polar.



(a) A bacterium running and tumbling. Notice that the direction of flagellar rotation (blue arrows) determines which of these movements occurs. Gray arrows indicate direction of movement of the microbe.



TEM 2 μm

(b) A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.

Figure 4.9 Flagella and bacterial motility.

Q Do bacterial flagella push or pull a cell?

- Bacterial flagellar motion= Rotatory in nature
- Necessary energy is derived from proton gradient that exists across the plasma membrane
- ATP is not directly required for flagellar motion
- Membrane proteins **MotA** and **MotB** creates a proton channel that derives the rotation of the flagellum
- **FlhG** is particularly important in generating flagellar rotation
- The rotation of flagella can be clockwise or counterclockwise
- When flagella rotates clockwise= forward motion ceases and the cells tumble

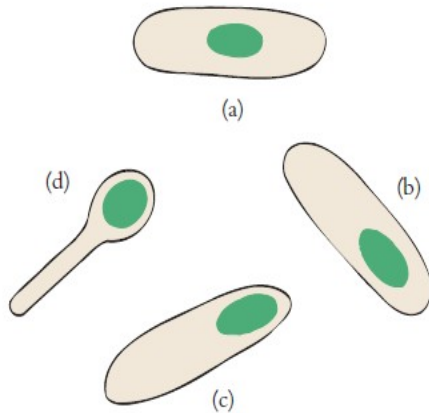


Figure 3.40 Examples of Endospore Location and Size. (a) Central spore. (b) Subterminal spore. (c) Terminal spore. (d) Terminal spore with swollen sporangium.

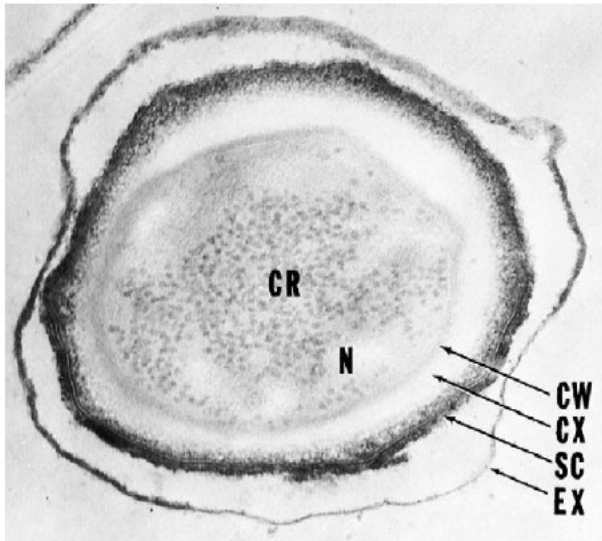


Figure 3.41 Endospore Structure. *Bacillus anthracis* endospore (×151,000). Note the following structures: exosporium, EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.

Endospores:

- The dormant structure which is heat resistant and dehydrated structure capable of surviving for long periods in an unfavourable environment
- It develops within the cell
- Bacteria in the genus ***Bacillus*** and ***Clostridium*** forms endospores
- Resistant to environmental stresses such as Heat, Ultraviolet Radiation, Gamma Radiation, Chemical Disinfectants and Desiccation

Endospore structure: many layers

1. **Exosporium**- thin delicate protein covering
2. **Spore coat**- lies beneath the exosporium composed of several protein layers
3. **Cortex**- present beneath the spore coat, made up of Peptidoglycan

Spore cell wall or core wall- Present beneath cortex and surrounds the protoplast or spore core which is Metabolically inactive

Endospore contains,

- Large amount of dipicolinic acid complexed with calcium ions
- Contributes endospore's heat resistance
- To determine the morphology of endospore-

Schaeffer-Fulton endospore stain is used; it involves heating of bacteria with malachite green that penetrates endospores. It gives the endospore green

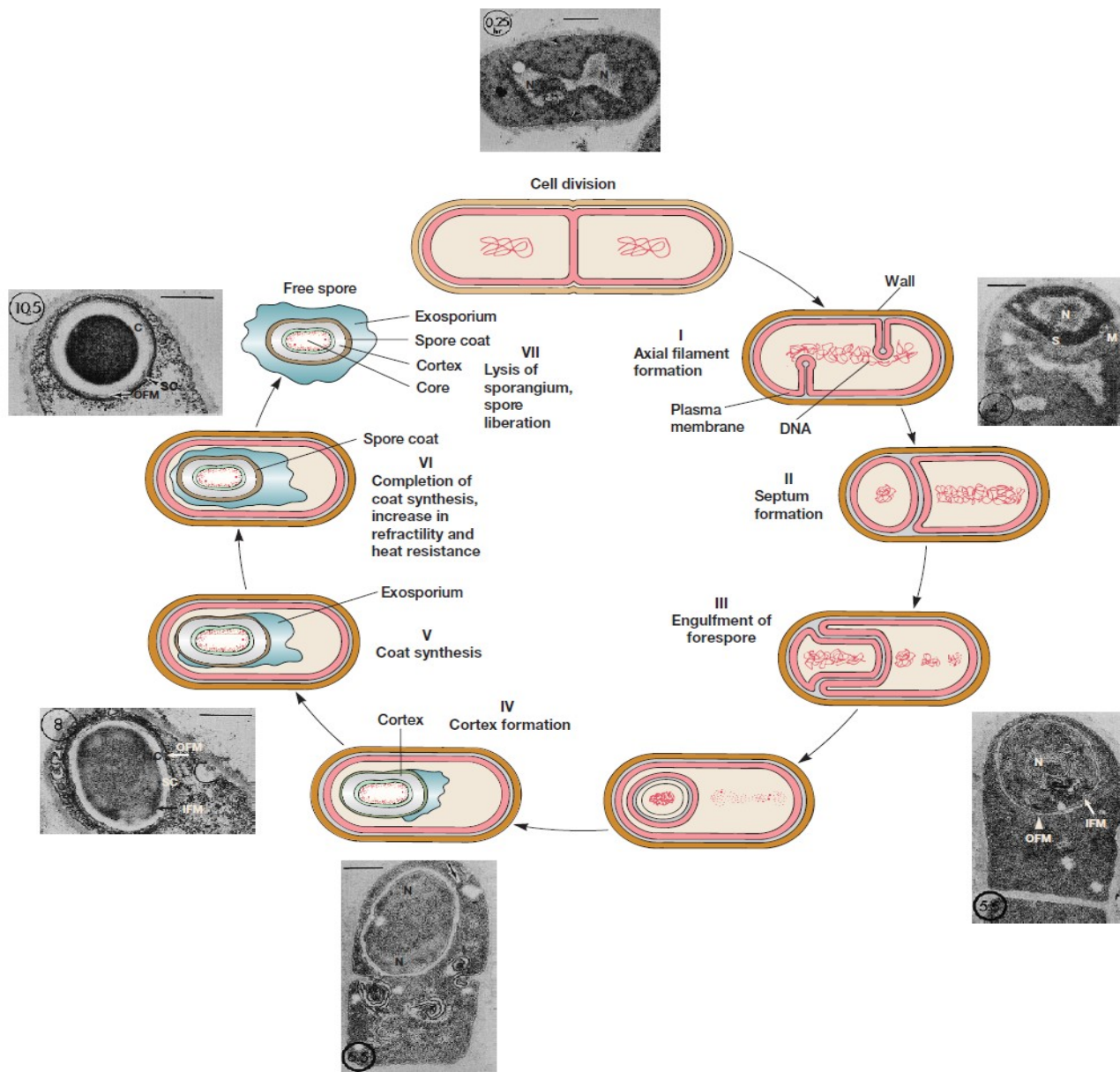
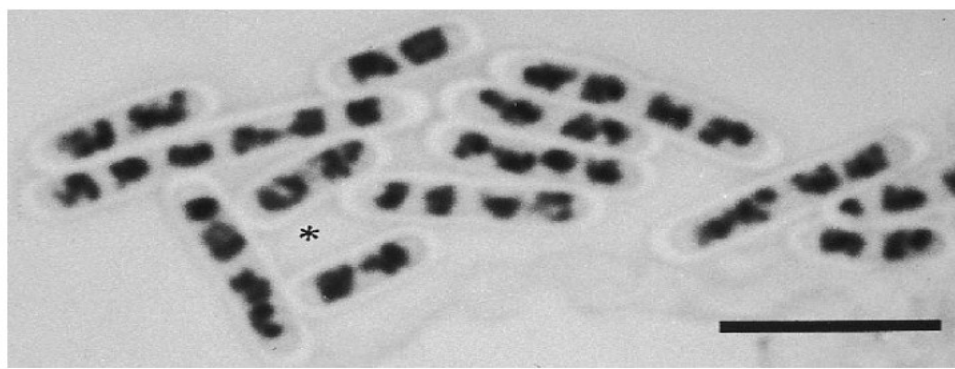


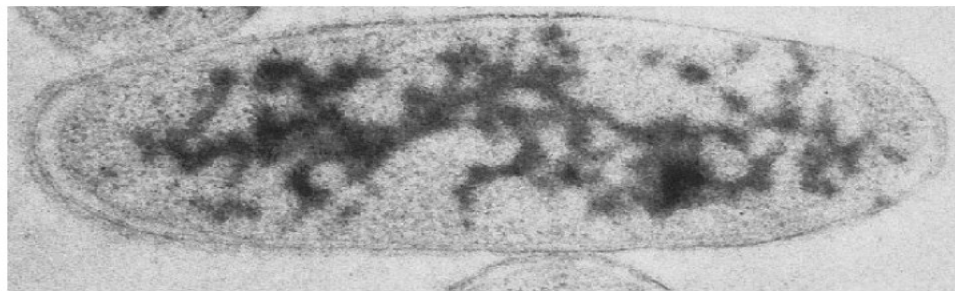
Figure 3.43 Endospore Formation: Life cycle of *Bacillus megaterium*. The stages are indicated by Roman numerals. The circled numbers in the photographs refer to the hours from the end of the logarithmic phase of growth: 0.25 h—a typical vegetative cell; 4 h—stage II cell, septation; 5.5 h—stage III cell, engulfment; 6.5 h—stage IV cell, cortex formation; 8 h—stage V cell, coat formation; 10.5 h—stage VI cell, mature spore in sporangium. Abbreviations used: C, cortex; IFM and OFM, inner and outer forespore membranes; M, mesosome; N, nucleoid; S, septum; SC, spore coats. Bars = 0.5 μ m.

Endospore formation:

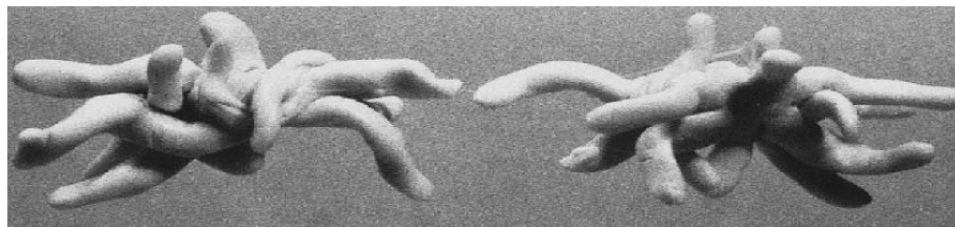
- When growing bacteria (the vegetative cell) is faced with starvation, the process of sporulation begins
- First morphological change is the transformation of two identical chromosomes (the parent chromosome and the newly replicated daughter chromosome) into an axial filament of DNA
- A septum then divides the cell and the smaller prespore cell is engulfed by the spore mother cell
- In this cell-within-cell, the plasma membranes separate the cytoplasm of one cell from the



(a)



(b)



(c)

Bacterial genome:

1. Nucleoid- Region in a bacterial cell that contains the bacterial chromosome

- Lacks a limiting membrane
- Bacterial chromosome is a circular (mostly) dsDNA molecule
- Does not contain basic histone proteins but associated with low-molecular-weight polyamines
- Bacterial chromosomes organized into independently supercoiled loops called **domains**
- Domain organization enables the chromosomal DNA to undergo structural changes during different cellular processes (Replication, Transcription and Segregation) that takes place simultaneously in a bacterial cell

Figure 3.14 The Bacterial Nucleoid. (a) Nucleoids in growing *Bacillus* cells stained using HCl-Giemsa stain and viewed with a light microscope (bar = 5 μm). (b) A section of actively growing *E. coli* immunostained specifically for DNA and examined in the transmission electron microscope. Coupled transcription and translation occur in parts of the nucleoid that extend out into the cytoplasm. (c) A model of two nucleoids in an actively growing *E. coli* cell. Note that a metabolically active nucleoid is not compact and spherical but has projections that extend into the cytoplasmic matrix.

2. Nucleoid- associated proteins:

- **Nucleoid- associated proteins** such as **HU (heat-Unstable nucleoid protein)**, **H-NS (Histone-like Nucleoid Structuring)**, **IHF (Integration Host Factor)** and **SMC (Structural Maintenance of Chromosomes)** facilitate compaction of chromosomal DNA by Bending, Bridging and Wrapping
 1. **SMC protein** also involved in Chromosome Segregation
 2. **H-NS protein** involved in condensation of DNA into the nucleoid
 3. **HU protein and IHF protein** involved in bending of DNA
- They help in Integration, Inversion and Recombination events by bending DNA

3. Chromosome and DNA molecule:

- Most bacteria like ***E. coli*** contain a single circular DNA molecule as a chromosome; some contain more than one chromosomes of linear or circular nature. Ex. ***Vibrio cholerae*** (bacterium that causes Cholera) contains two circular chromosomes
- One of three chromosomes contains the genes involved in metabolism and virulence, while other contains the remaining essential genes
- Ex. ***Borrelia burgdorferi*** (Bacteria that causes Lyme disease) contains upto 11 copies of a single linear chromosome
- Archaeans are the only group of prokaryotes that use eukaryote-like histones to condense their DNA molecule
- DNA molecules of Archaea can also be negatively supercoiled, positively supercoiled, or not supercoiled at all

4. Plasmids:

- Term coined by J Lederberg
- Autonomous self-replicating molecules of DNA that are maintained as discrete, extrachromosomal genetic elements in bacteria
- It is a replicon (replicon is self-replicating molecule of nucleic acid)
- Plasmids are mostly DNA molecules; RNA plasmids (both single stranded and double stranded form) are very rare and reported in few plants, fungi and even in animals
- Bacterial plasmids are much smaller than the bacterial chromosome, varying from less than 5kbp to more than several hundred kbp, though plasmids are large as 2Mbp also occur in some bacteria
- They usually encode traits that are not essential for bacterial viability and replicate independently of the chromosome
- Most plasmids are circular, negatively supercoiled, dsDNA molecules, but linear have also been reported in genera ***Borrelia*** and ***Streptomyces***
- **Functions of plasmids:**
- Plasmids encode many different proteins; they rarely encode gene products that are essential for growth, such as RNA polymerase, ribosomal subunits or enzymes of tricarboxylic acid cycle
- Genes encoded by plasmids include enzymes for the utilization of unusual carbon sources such as toluene, resistance to substances such as heavy metals and antibiotics, synthesis of antibiotics and synthesis of toxins and proteins that allow the successful infection of higher organisms
- Plasmid that confers no identified functions or phenotypic properties is termed as **Cryptic Plasmid**

Conjugative plasmids:

- Self-transmissible plasmid
- It possesses all the necessary genes to transmit itself to another bacterium by conjugation
- It contains both **Mpf** genes (involved in mating pair formation) and **Dtr** genes (involved in DNA transfer and replication)
- **Mpf** genes encode proteins responsible for pilus-mediated cell-to-cell contact and formation of Type IV secretion system (**T4SS**) which acts as a channel through which proteins or protein-DNA complexes can be translocated
- **Dtr** genes encode proteins that bind to the DNA at the origin of transfer **oriT**, forming a complex called **relaxosome**
- The key catalytic component of the relaxosome is the **relaxase** (which produces a nick within **oriT** and becomes transiently attached to the 5' end of the DNA single strand)

• Ex. F plasmids

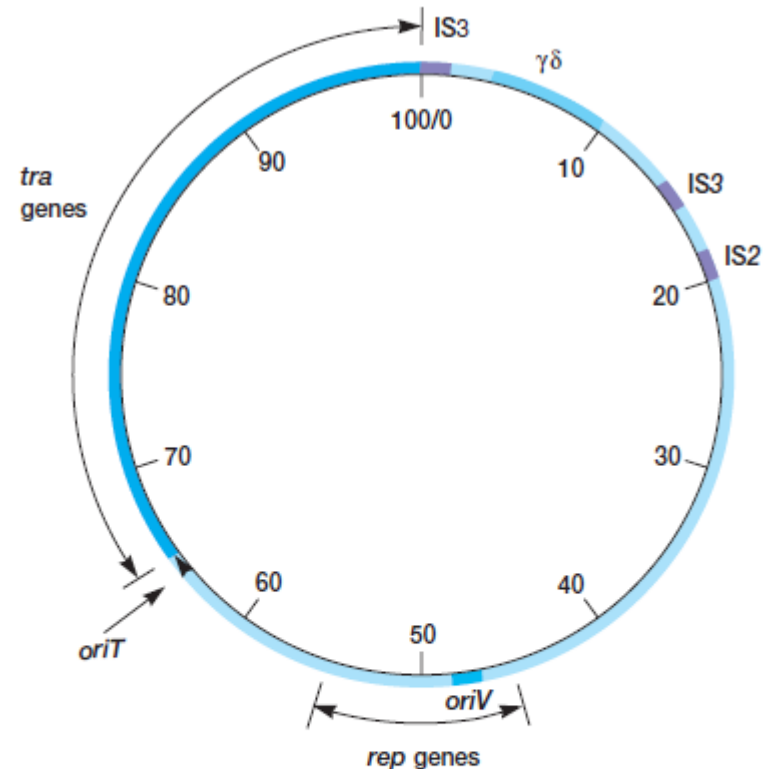


Figure 13.5 The F plasmid. A map showing the size and general organization of the F plasmid. The plasmid contains several transposable elements. IS2 and IS3 are insertion sequences; $\gamma\delta$ is also called transposon Tn1000. The *tra* genes code for proteins needed in pilus synthesis and conjugation. The *rep* genes code for proteins involved in DNA replication. *OriV* is the initiation site for circular DNA replication and *oriT*, the site for initiation of rolling circle replication and gene transfer during conjugation.

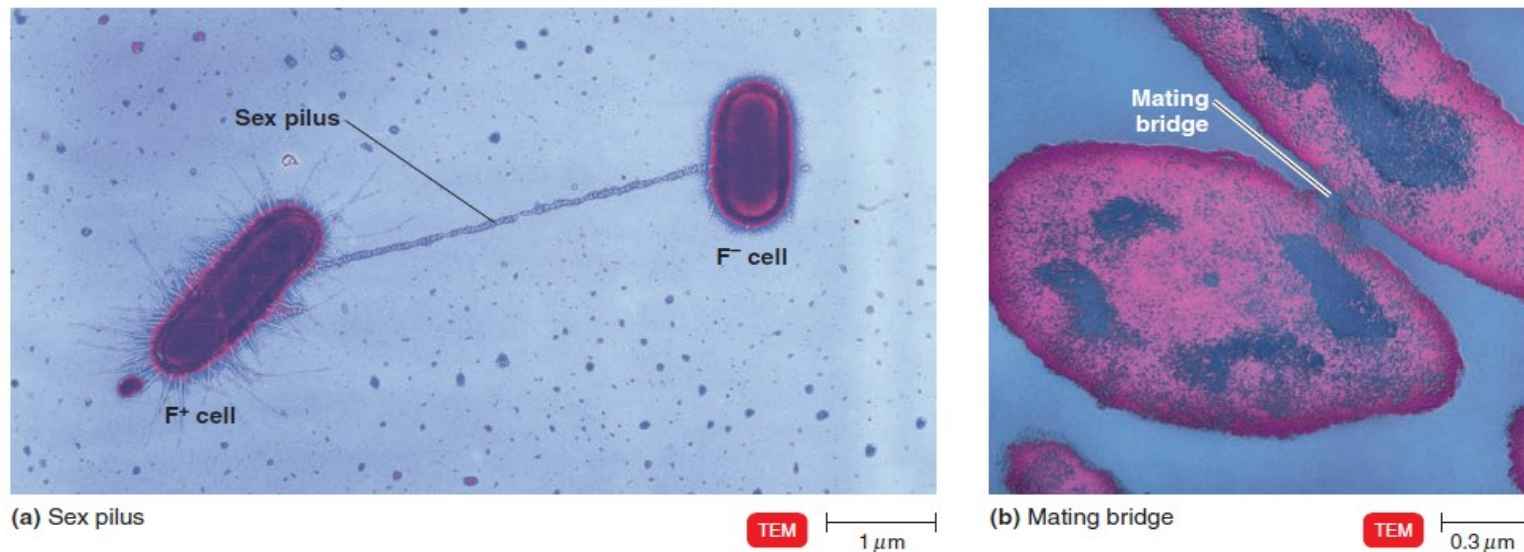


Figure 8.27 Bacterial conjugation.

- Bacteria that possess a copy of F-plasmid are called **F-positive** or **F-plus** (denoted **F⁺**)
- Cells that lack F- plasmids are called **F-negative** or **F-minus** (**F⁻**)
- This plasmid is also an **Episome** (can exist autonomously in a cell or can integrate itself into the bacterial chromosome)
- Bacterial cell that contains an F- plasmid integrated with the bacterial chromosome is referred to as an **Hfr (High frequency recombination)** cell
- Integration involves homologous recombination between two covalently closed circular DNA molecules forming one circular molecule containing both of the original DNA structures
- It is thought that the insertion sequences (**IS sequences**) present in the F-plasmid and those in the host chromosome serve as regions of homology for the

Mobilizable plasmids:

- Possesses a functional ***oriT*** and ***Dtr*** genes for DNA transfer and replication
- It carries the genes necessary for relaxosome formation and DNA transfer, but lack the genes required for mating pair formation
- These plasmids are not able to promote their own transfer unless an appropriate conjugation system is provided by a conjugative plasmid

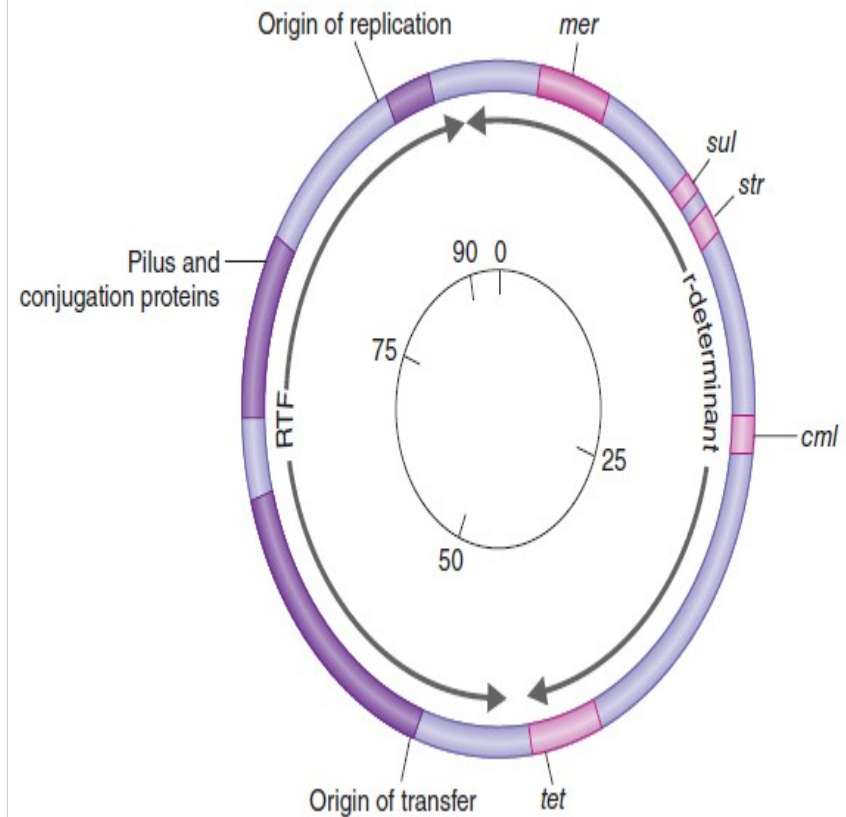
Ex. **R-plasmids**

- R-plasmid make the host cell resistant to one or more antibiotics
- They typically have genes that code for enzymes capable of destroying or modifying antibiotics
- Some R-plasmids have only a single antibiotic resistance gene, whereas others have many

Col plasmids

Colicinogenic plasmids

- These plasmids synthesize proteins termed as **bacteriocin** that prevent the growth of susceptible bacterial strains that do not contain a Col plasmid
- Bacteriocins are proteins that inhibit the growth of other closely related bacterial strains
- Bacteriocins produced by ***E. coli*** are termed colicins which have a particular mode of inhibition of sensitive cells



Properties of plasmids:

- **Plasmid replication-** Plasmids use 2 alternative mechanisms for replicating their DNA

1. Most plasmids replicate like bacterial chromosomes

- They have an origin of replication where the DNA opens and bidirectional replication begins
- 2 replication forks move around the circular plasmid in opposite directions until they meet

2. The other replication mechanism is Rolling Circle Replication

- It starts by nicking at the origin of replication and unrolling one strand and using the other, still circular strand as a template for DNA synthesis
- Some plasmid (F-plasmid) of ***E. coli*** can transfer themselves from one bacterium to another- such plasmids have 2 separate origin of replication
- Bidirectional replication (also known as vegetative replication) starts at **oriV** and transfer starts from **oriT**
- All plasmids must have a vegetative origin since they all replicate to survive
- But only those plasmids that can transfer themselves have the origin of transfer

- **Copy number-** The average number of molecules of a given plasmid per bacterial cell is called its copy number (it is the number of copies of plasmid in a cell immediately after cell division)
 - Plasmids are classified into **Low Copy Number** or **Stringent Plasmids** (with 1 to 2 copies per cell) and **High Copy Number** or **Relaxed Plasmids** (with 10 to 100 copies per cell)
 - Each type of plasmid possesses its own genes for regulation of replication initiation and hence the number of plasmid copy per cell
 - Maintenance of copy number is by negative regulatory systems in bacteria that adjust the rate of replication of plasmid
 - The initiation of plasmid replication may be controlled by regulating the amount of available primer for the initiation of DNA replication, regulating the amount of essential replication proteins or regulating the function of essential replication proteins
 - 3 major mechanisms are used to control the initiation of plasmid replication:
 - Regulation by antisense RNA
 - Regulation by binding of replication proteins to repeated 18-22 bp sites called **iterons**
 - Regulation by ctRNA (Counter-transcribed RNA, a plasmid

- **Plasmid incompatibility-** meaning the inability of two closely related plasmids to stably coexist in the same cell
 - 2 plasmids that cannot coexist in the same bacterial cell are members of the same incompatibility group i.e. two plasmids are incompatible if they belong to the same incompatibility group
 - Incompatible plasmids share same replication control mechanism
 - Thus the replication control system will not recognize the two incompatible plasmids as different, and so either may be randomly selected for replication
- **Partitioning-** It is an active process which assures that after cell division each daughter cell gets a copy of plasmid
 - For plasmids present in high copy numbers (50 to 100 copies per cell), random diffusion may be enough to get at least one copy of the plasmid to each daughter cell
 - Random segregation of low copy number plasmids (only 1 to 2 copies per cell) would most likely mean that, following cell division, one of the daughter cells would not receive a plasmid
 - The plasmid would eventually be diluted from the population
 - Consequently, regulated partitioning mechanisms are essential for these plasmids:
 - Partitioning of low copy number plasmids- regulated by ***par*** genes present on plasmids
 - The ***par*** systems consist of cis-acting Centromere-like site, often

Horizontal gene transfer and genetic recombination:

- Gene transfer- Movement of genetic information between organisms
- Can be horizontal or vertical
- Transfer of genes from parents to offsprings= **Vertical Gene Transfer**
- Transfer of genes between two independent organisms= **Horizontal** or **Lateral Gene Transfer**
- 3 mechanisms of horizontal gene transfer:
 - 1. Transformation**
 - 2. Transduction**
 - 3. Conjugation**

- The fragment of DNA that has been transferred during horizontal gene transfer from a donor cell to a recipient cell = **Exogenote**
- The recipient bacterial cell's own genetic material into which the donor DNA can integrate = **Endogenote**
- A bacterial cell that has received an exogenote is initially diploid for parts of its genome= **Merozygote** (partial diploid/ merodiploid)

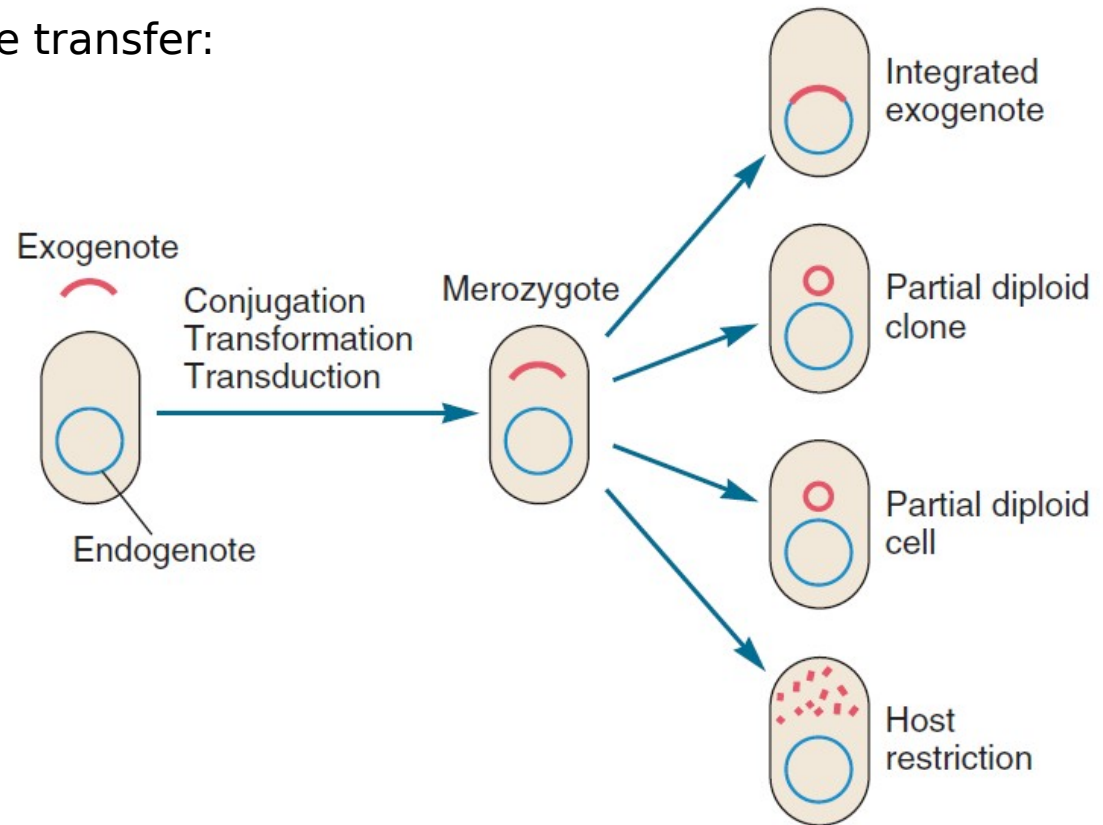


Figure 13.4 The Production and Fate of Merozygotes. See text for discussion.

Transformation

- First horizontal gene transfer mechanism discovered in bacteria
- It was discovered by Fred Griffith in 1928
- Uptake of a naked DNA molecule or fragment from the medium by a cell and the incorporation of this molecule into the chromosome of the recipient
- This process may be natural or artificial
- **Natural transformation**- very rare event; observed in Gram-Positive (***Streptococcus pneumoniae***, ***Bacillus subtilis***) as well as Gram-Negative bacteria (***Haemophilus influenzae***)
- Ability of a recipient bacterium to take up free DNA and become transformed=**Competence** (competence- is an inheritable characteristics of certain bacterial strains)
- Competent bacteria that can take up DNA encode protein called **Competence**

Mapping by transformation

- Mapping by transformation facilitate binding of DNA fragments to cell surface and uptake of DNA into the cytoplasm
- **Principle**: 2 genes transform together if they are near enough to be carried on the same DNA fragment
- Consider 2 genes **A** and **B** present on the bacterial chromosome
 - If 2 genes are widely separated in the chromosome= they are always carried on two different DNA fragments= the probability of simultaneous transformations of the A-B-recipient is very less
 - If 2 genes are near one another= they are often present on a single DNA fragment= frequency of simultaneous transformations (co-transformation) is same as the frequency of single gene transformation
- Thus frequency of co-transformation is inversely proportional to the distance between two genes

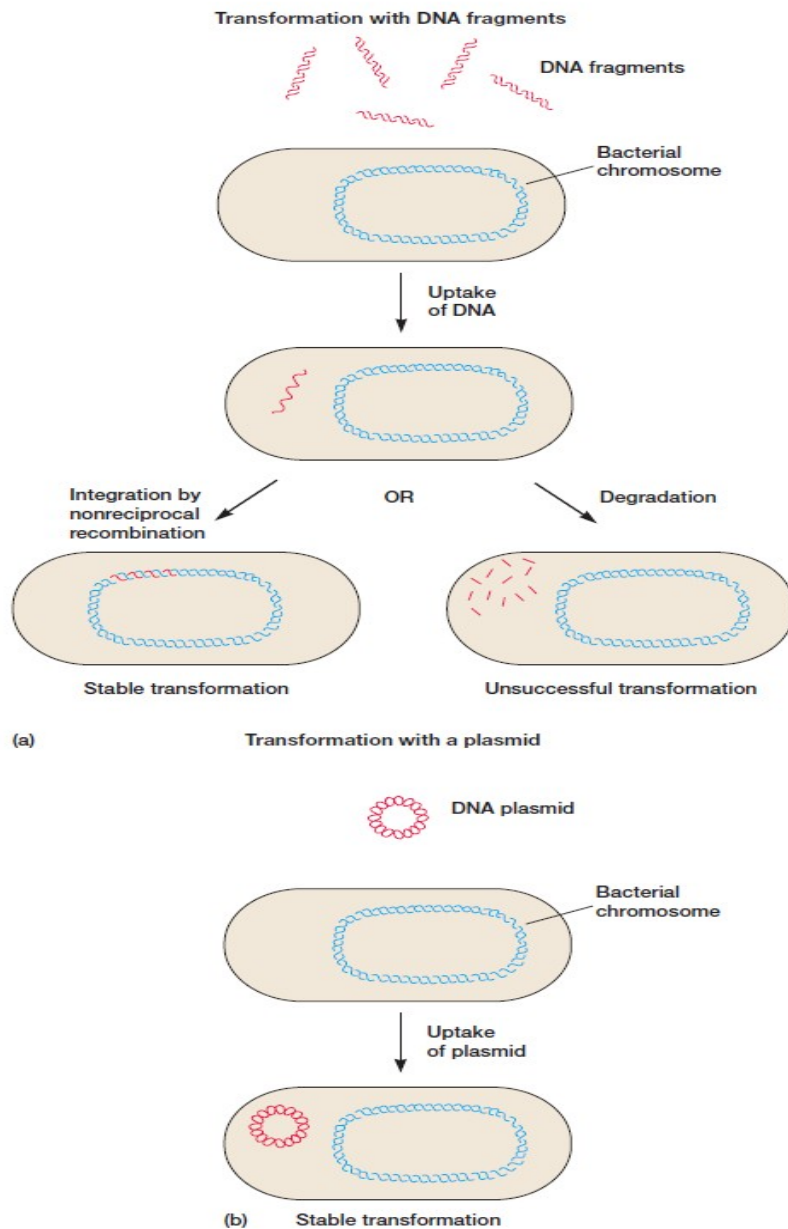


Figure 13.16 Bacterial Transformation. Transformation with (a) DNA fragments and (b) plasmids. Transformation with a plasmid often is induced artificially in the laboratory. The transforming DNA is in red and integration is at a homologous region of the genome. See text for details.

Process:

Naturally competent bacteria actively pull DNA fragments from their environment into their cells

DNA can be naturally released into the environment when bacterial cells die and lysis

Free DNA comes in contact with competent bacteria

Linear dsDNA enters into the cell

One strand is translocated across the membrane into the cytoplasm, with its 3' end leading

The strand not translocated, is degraded at membrane surface by nuclease

Translocated strand integrates with chromosome by homologous recombination

Recipient cells that undergo this process and acquire a new phenotype as a result are said to be transformed

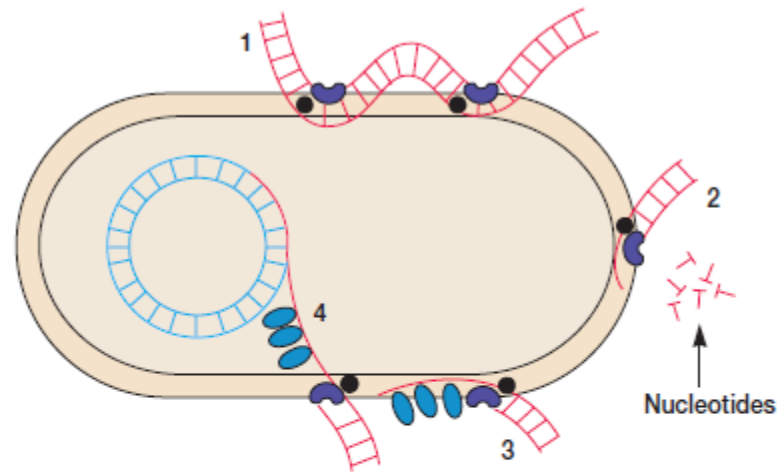


Figure 13.17 The Mechanism of Transformation. (1) A long double-stranded DNA molecule binds to the surface with the aid of a DNA-binding protein (●) and is nicked by a nuclease (☾). (2) One strand is degraded by the nuclease. (3) The undegraded strand associates with a competence-specific protein (●). (4) The single strand enters the cell and is integrated into the host chromosome in place of the homologous region of the host DNA.

Transduction

- This was first discovered by Lederberg and Zinder in 1951 in ***Salmonella typhimurium***
- In this process, bacteriophage functions as vector to transfer DNA from donor bacteria into recipient bacteria

2 types of transduction:

1. Generalized transduction
2. Specialized transduction

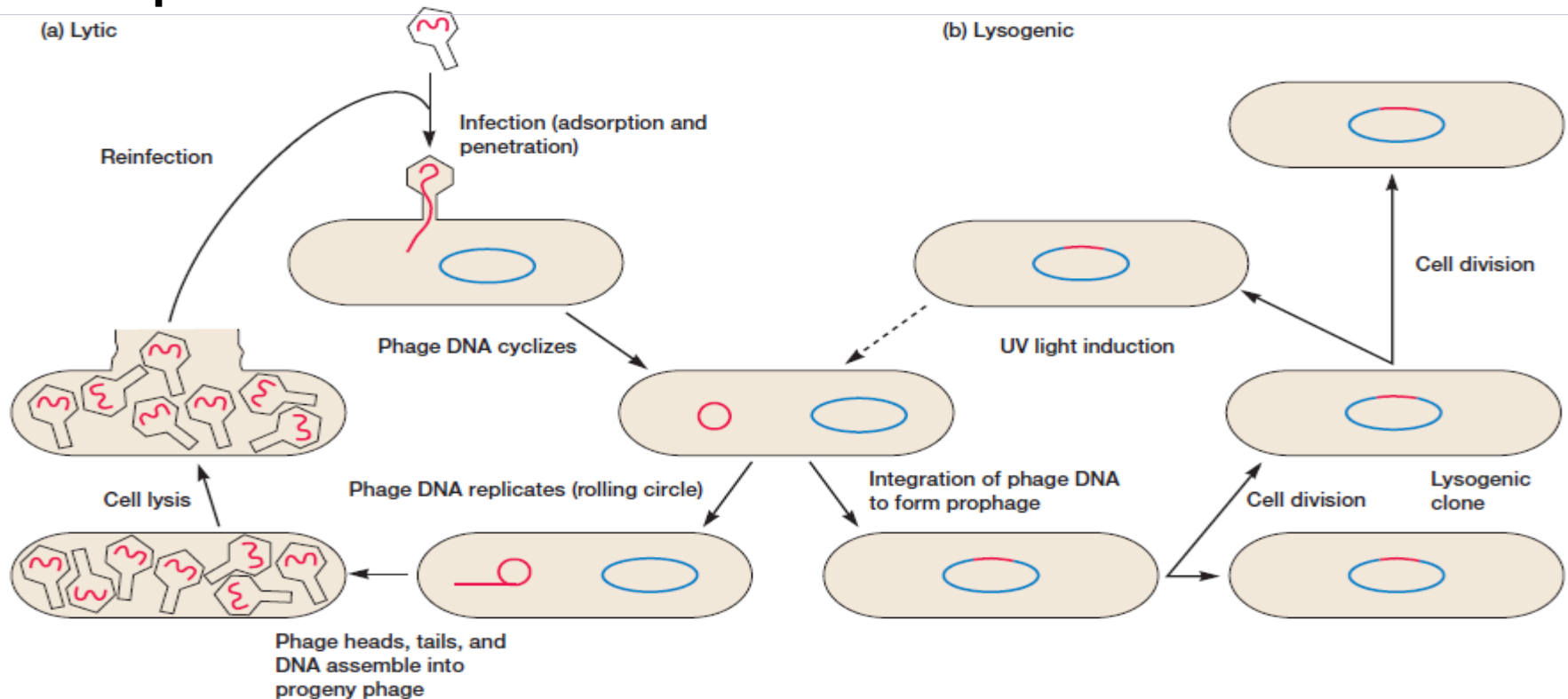
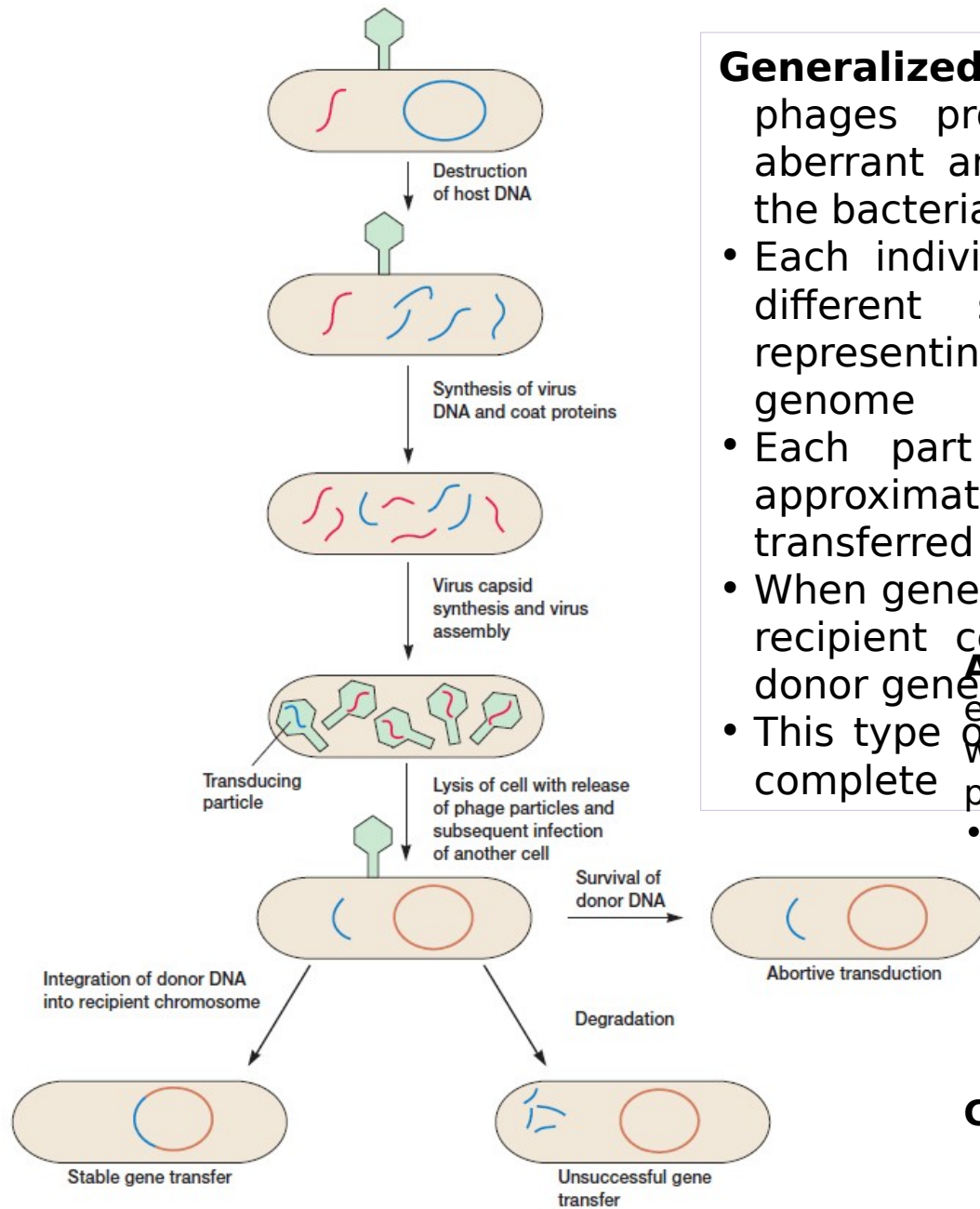


Figure 13.18 Lytic versus Lysogenic Infection by Phage Lambda. (a) Lytic infection. (b) Lysogenic infection. Viral and prophage DNA are in red.



Generalized transduction: Transducing phages produced during lytic growth are aberrant and contain a random fragment of the bacterial genome instead of phage DNA

- Each individual transducing phage carries a different set of closely linked genes, representing a small segment of the bacterial genome
- Each part of the bacterial genome has approximately the same probability of being transferred from donor to recipient bacteria
- When generalized transducing phage infects a recipient cell, expression of the transferred donor genes occurs
- This type of transduction may be abortive or complete

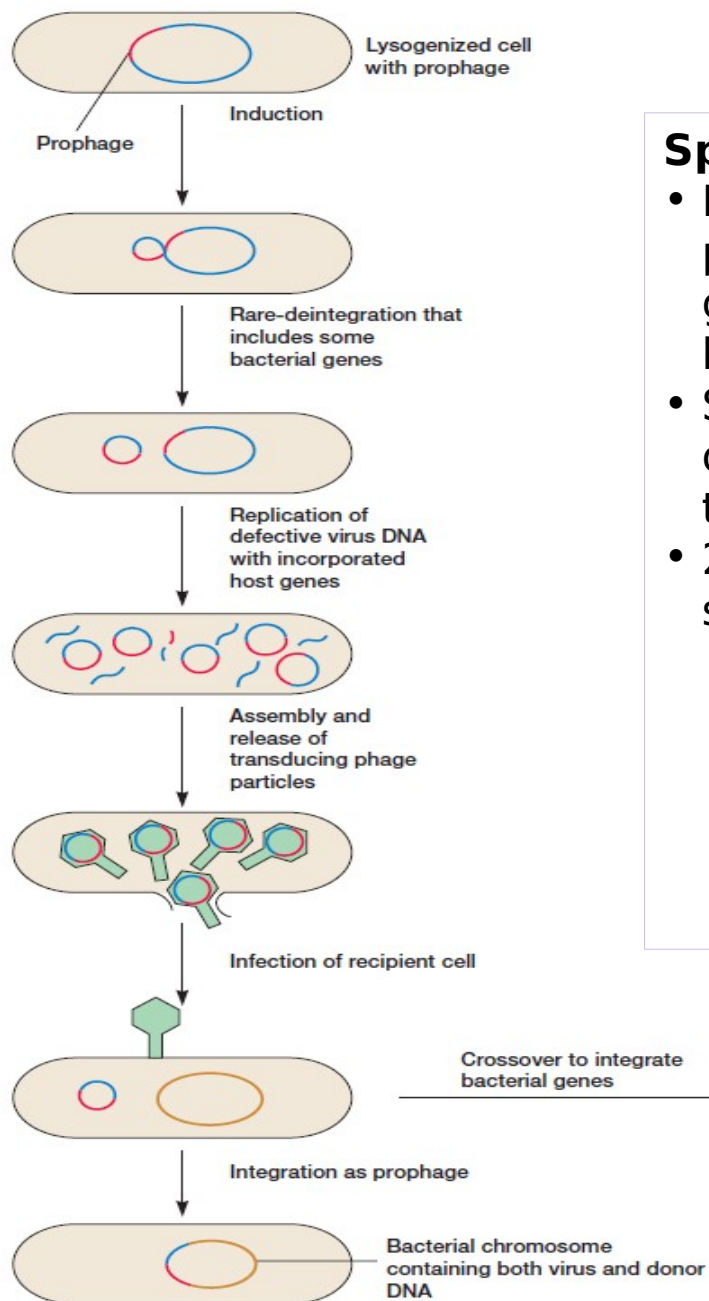
Abortive transduction: Transient expression of one or more donor genes without formation of recombinant progeny

- The donor DNA fragment does not integrate with endogenote and also not replicate, and among the progeny of the original transductant only one bacterium contains the donor DNA fragment

Complete transduction: production of stable recombinants that inherit donor genes and retain the ability to express them

- DNA remain double stranded during

Figure 13.19 Generalized Transduction by Bacteriophages. See text for details.



Specialized transduction:

- Mediated only by specific temperate phages and only a few specific donor genes can be transferred to recipient bacteria
- Specialized transducing phages are formed only when lysogenic donor bacteria enter the lytic cycle and release phage progeny
- 2 distinguishing characteristics of specialized (restricted) transduction:
 - Only bacterial genes that can be transduced are those very near the site at which the Prophage is integrated
 - It results from defective excision of the Prophage from the host chromosome

Figure 13.20 Specialized Transduction by a Temperate Bacteriophage. Recombination can produce two types of transductants. See text for details.

- They lack part of the normal phage genome and contain part of the bacterial chromosome located adjacent to the Prophage attachment site
- Formation of the λ **gal** and λ **bio** transducing particles causes loss of some λ genes
 - λ **gal particles**- lack the tail genes and sometimes head genes both of which are located at the right end of Prophage
 - λ **bio particles**- lacks genes from the left end of the Prophage (**int, xis**)
 - The head and tail genes are essential, **so** λ **gal** transducing particles are unable to perform lytic cycles and thus plaques
 - They are defective and this is denoted λ **dgal**
 - The genes missing in bio transducing particles are not essential for lytic growth, so λ **bio** phages are usually plaque forming and are called λ **pbio** (p means plaque forming)
 - The transducing particle (λ **dgal** which lacks phage genes required to grow lytically) is produced by aberrant excision from a normal Prophage
 - The Prophage contains all of the essential genes and hence the necessary gene products (head and tail proteins) are still present in the chromosome
 - If a bacterial cell is double infected with a wild type lambda phage and a λ **dgal** phage, the wild type phage can supply the functions missing in the defective phage and the progeny will contain about equal number of both types
- Specialized transducing phages are formed by aberrant excision of prophage (rare event)
- When the aberrant excision initially occurs, It will yield less than one specialized transducing phage per 1,000,000 wild type phage
- Thus this lysate is called **Low Frequency Transducing Lysate (LFT)**
- If this specialized transducing phage co-infects a new host together with a wild type phage, a dilyso-gen can result
- Induction of dilyso-gen yields a lysate containing 50% specialized transducing phage and 50% wild type phage

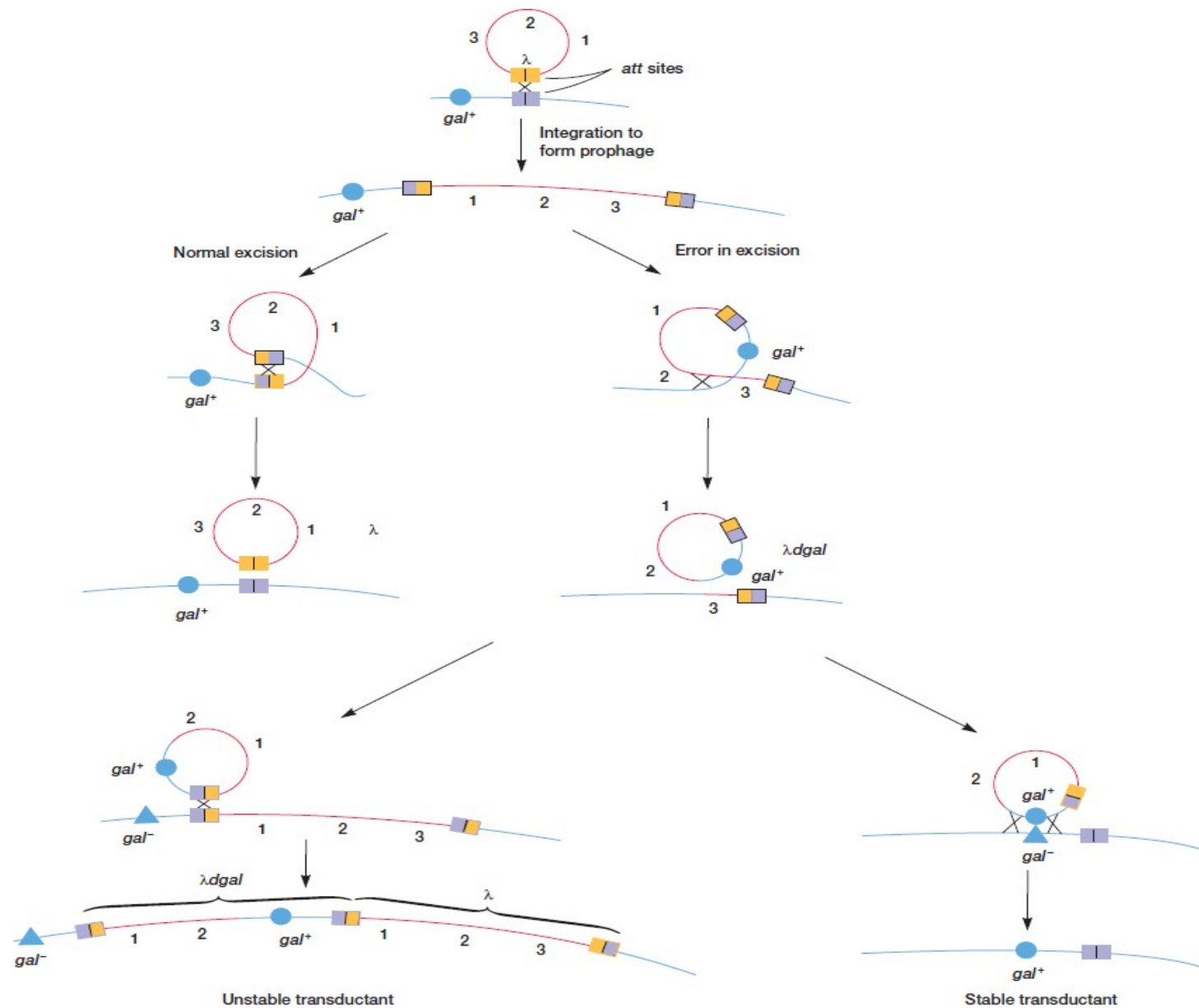
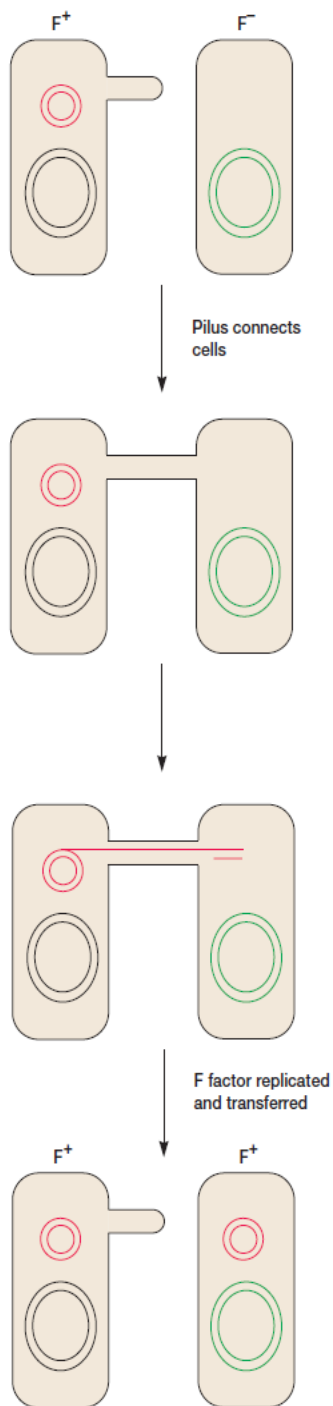


Figure 13.21 The Mechanism of Transduction for Phage Lambda and *E. coli*. Integrated lambda phage lies next to the *gal* genes. When it excises normally (top left), the new phage is complete and contains no bacterial genes. Rarely excision occurs asymmetrically (top right), and the *gal* genes are then picked up and some phage genes are lost. The result is a defective lambda phage that carries bacterial genes and can transfer them to a new recipient. See text for details.

Generalized transduction	Specialized transduction
Occurs during the lytic of virulent and temperate phage	Occurs during the lysogenic cycle of temperate phage
Phages penetrate the bacterial cell and enter lytic cycle	Phages penetrate the bacterial cell and enter lysogenic cycle
Viral DNA begins to replicate immediately the bacterial cytoplasm	Viral DNA integrates into the DNA of the bacterium in and replicates later
Any bacterial genes are randomly packaged into new phage particles	Bacterial genes adjacent to previously incorporated virus are packaged into new phage particles
Some new phages have no viral DNA	Some new phages have both viral and bacterial DNA
Donor bacterial genes are incorporated into chromosome of new bacterium	Donor bacterial genes are incorporated into chromosome of new bacterium together with genome of transducing phages

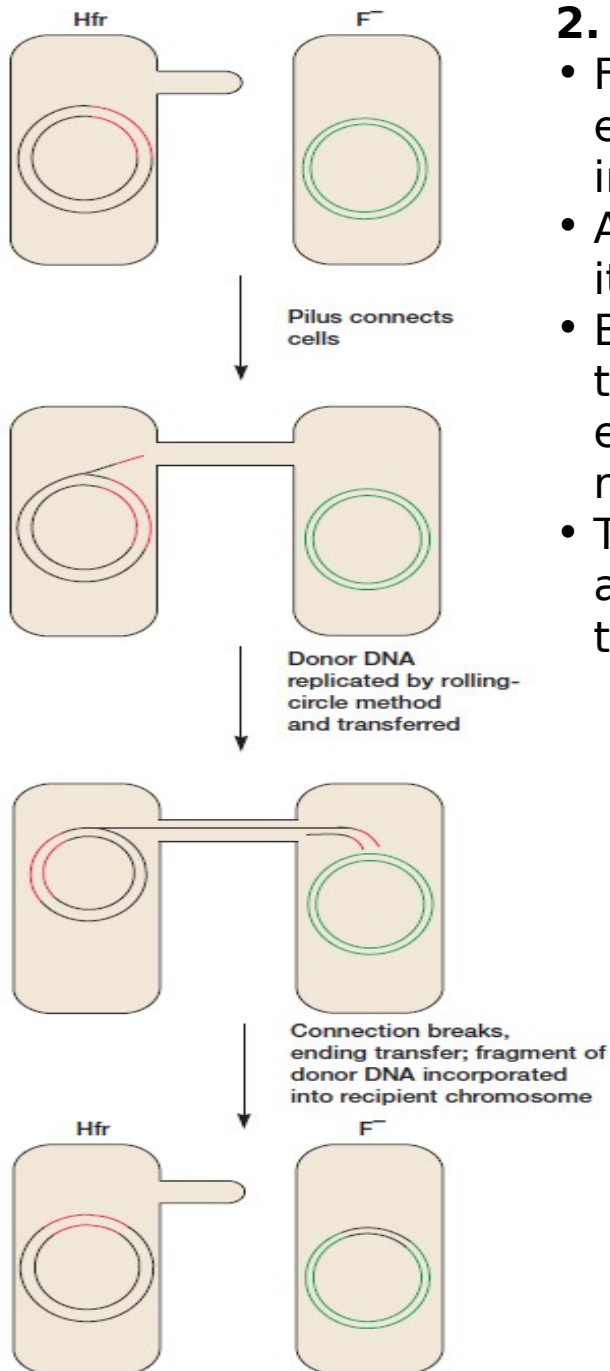
Conjugation:

- Process in which the genetic information from one bacteria is transferred to and recombined with that of other bacteria
- Direct contact between the donor and recipient bacteria leads to the establishment of a cytoplasmic bridge between them and transfer of a part or all the donor genome to the recipient takes place
- Donor ability= presence of self transmissible/ conjugative plasmids called **fertility plasmids** or **sex plasmids**
- Recipient cell that has received DNA as a result of conjugation = **Transconjugant**
- Transfer of plasmids is generally intraspecific
- 3 types of conjugations are mentioned:
 1. **F⁺- F⁻ conjugation**
 2. **Hfr-F⁻ conjugation**
 3. **F' -F⁻ conjugation**



1. F⁺- F⁻ conjugation:

- F plasmid (F-factor) of *E. coli* is the prototype for fertility plasmids in Gram-Negative bacteria
- Strains of *E. coli* with an extrachromosomal F plasmid = **F⁺** = functions as **Donors**
- Strains of *E. coli* that lack the F plasmid = **F⁻** = functions as **Recipients**
- Conjugative functions of the plasmid are specified by a cluster of at least 25 transfer (**Tra**) genes which determine the expression of sex pili, synthesis and transfer of DNA during mating, interference with the ability of **F⁺** bacteria to serve as recipients and other functions
- **Tra** genes divided into 2 groups:
 - Group 1- whose products are involved in the mating pair formation (**Mpf**)
 - Group 2- whose products are involved in processing the plasmid DNA for transfer (**Dtr**)
- The **Mpf** component includes a sex pilus that extrudes from the cell and holds mating cells together
- The **Mpf** system also includes a **type IV secretion system (T4SS)** which act as a channel through which DNA and protein pass
- Each F⁺ bacterium has 1 to 5 sex pili that bind to a specific outer membrane protein on recipient bacteria to initiate mating
- Intercellular cytoplasmic bridge is formed
- One strand of the F plasmid DNA is transferred from donor to recipient, beginning at a unique origin and progressing in the 5' to 3' direction
- The site on the plasmid DNA at which transfer initiates is called the origin of transfer (**oriT**)
- **Relaxase** protein makes a single strand cut at the **oriT** site in the plasmid
- The transferred strand is converted to circular double-stranded F plasmid DNA in the recipient bacterium
- Both of the exconjugant bacteria are **F⁺** and the F plasmid can



2. Hfr-F⁻ conjugation:

- F plasmid in *E. coli* can exist as an extrachromosomal genetic element or be integrated into the bacterial chromosome
- An *E. coli* strain with an integrated F plasmid retains its ability to function as a donor in conjugal matings
- Because donor strains with integrated F plasmids can transfer chromosomal genes to recipients with high efficiency, they are called **Hfr** (High frequency recombination) strains
- Transfer of single-stranded DNA from an **Hfr** donor to a recipient begins from the origin of transfer within the F plasmid and proceeds
 - In matings between **F⁺** and **F⁻** bacteria, only the F plasmid is transferred to recipients
 - In case of matings between **Hfr** and **F⁻** strains, part of the F plasmid is transferred last, after the entire bacterial chromosome has been transferred
 - Because only a part of the F plasmid is transferred at the start, the **F⁻** does not become **F⁺** unless the whole chromosome is transferred
 - Complete transfer takes about 100 minutes in *E. coli*, and the conjugation usually breaks before this process is finished
 - Thus a complete F plasmid usually is not transferred and the recipient remains **F⁻**
 - After the replicated donor chromosome enters the recipient cell, it may be degraded or incorporated into the **F⁻** genome by recombination

3. F' -F⁻ conjugation:

- Integrated F plasmids in **Hfr** strains can sometimes be excised from the bacterial chromosome
- If excision precisely reverses the integration process, **F⁺** cells are produced
- On rare occasions, however, excision occurs by recombinations involving insertion sequences or other genes on the bacterial chromosome that are located at some distance from the original integration site
- In such cases segments of the bacterial chromosome can become incorporated into hybrid F plasmid that are called **F'** plasmids
- **F'-F⁻** conjugation is virtually identical with **F⁺-F⁻** conjugation
- Bacterial genes on the **F'** plasmid are transferred with it and need not be incorporated into the recipient chromosome to be expressed
- The recipient becomes **F'** and is partially diploid merozygote since it has two sets of the genes carried by the plasmid

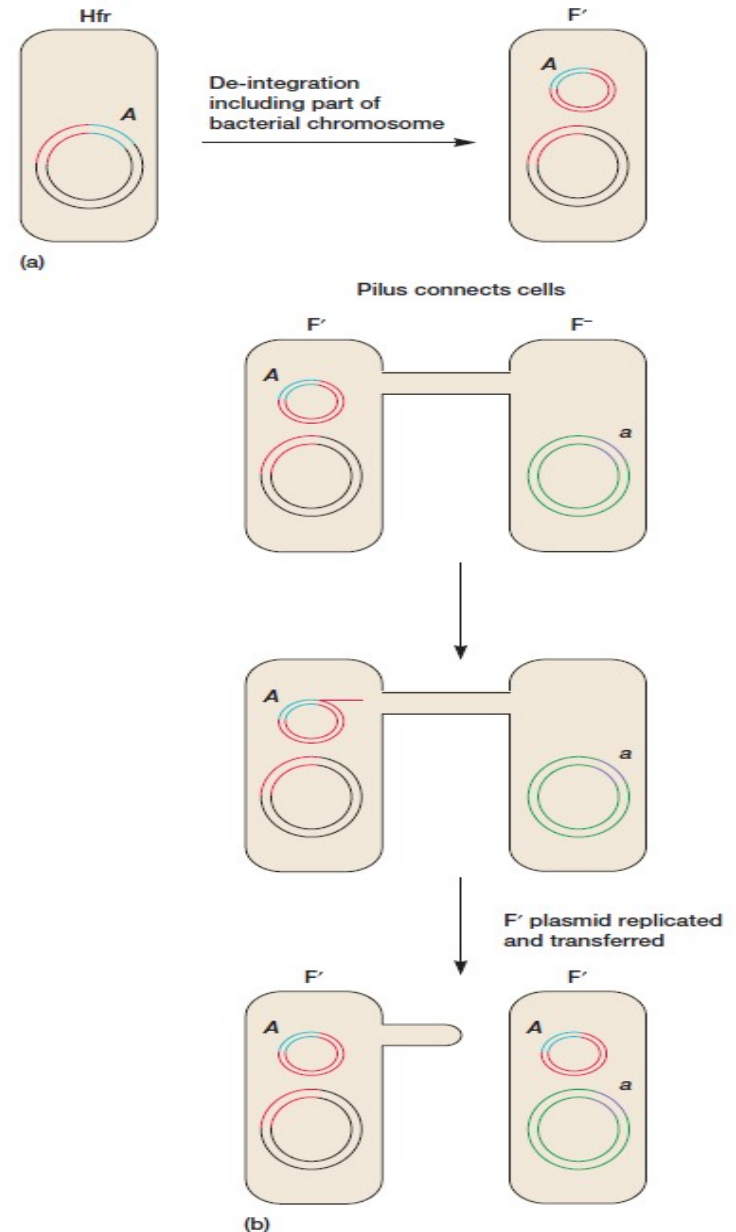
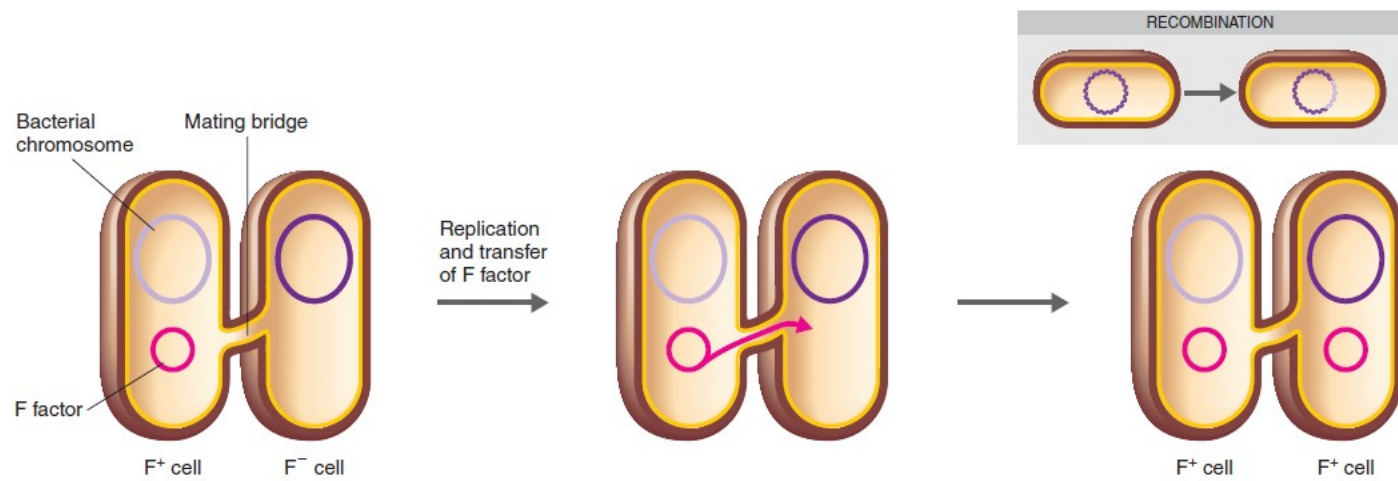
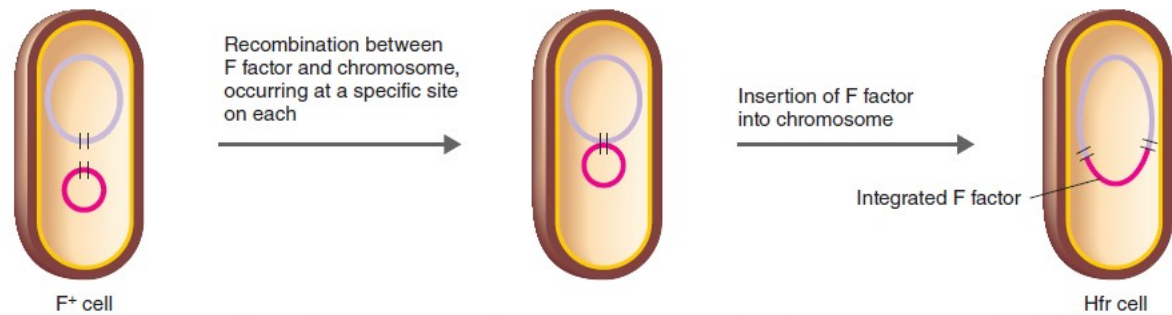


Figure 13.15 F' Conjugation. (a) Due to an error in excision, the A gene of an Hfr cell is picked up by the F factor. (b) The A gene is then transferred to a recipient during conjugation.



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

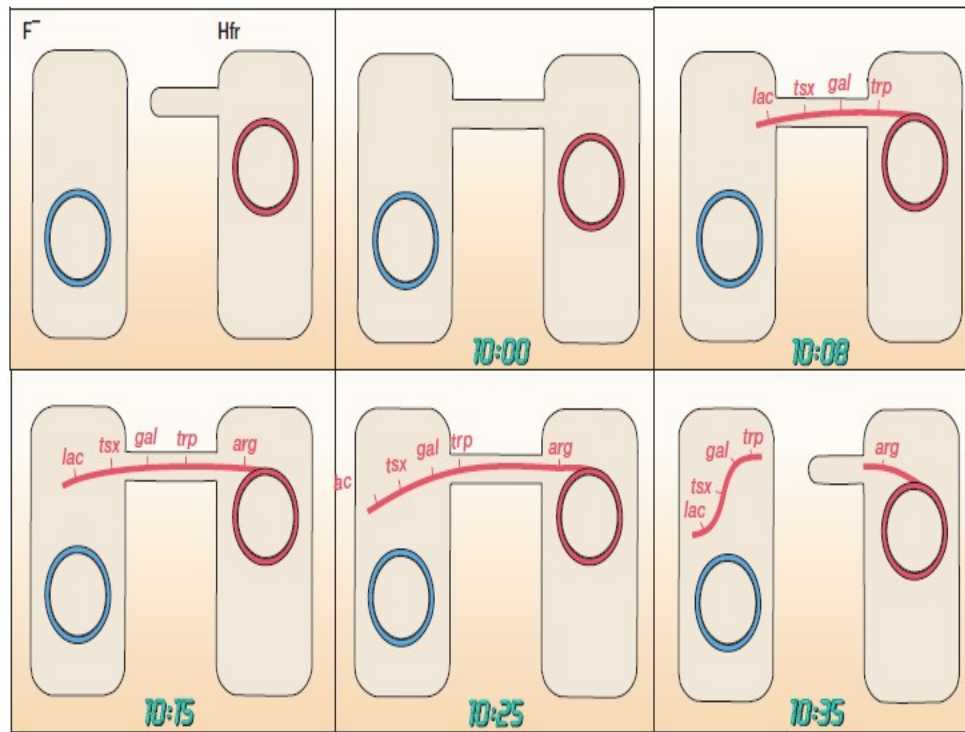


(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

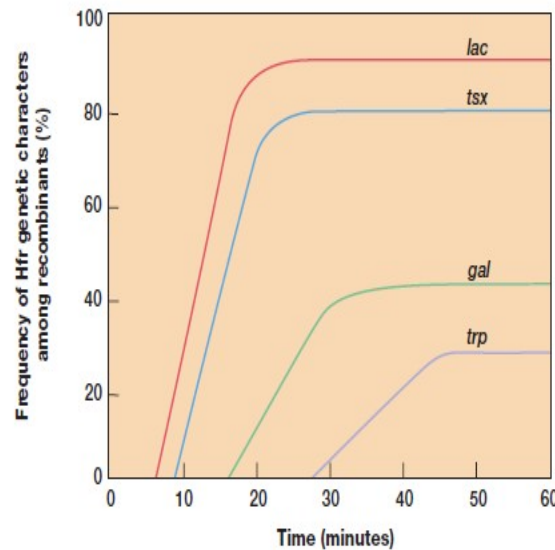
Figure 8.28 Conjugation in *E. coli*.

Interrupted mating experiment:

- Conducted by E Wollman and F Jacob
- **Principle:** Conjugation between different **Hfr** and **F⁻** strains can be used to map the relative positions of genes in the bacterial chromosomes. Genes closer to the origin of transfer will be transferred at a higher frequency than genes farther away from the origin
- **Procedure:**
 - Mixing of **Hfr** and **F⁻** strains with suitable marker gene: culture containing a mixture of **Hfr** and **F⁻** strain was incubated
 - Samples were removed at various intervals and each was placed in an agitating kitchen blender
 - Agitation in blender separates the conjugating bacteria and thus transfer of DNA
 - Assessment of the recombination of specific genes at different time: the cells were then allowed to grow on medium containing antibiotic in order to ensure the recovery of only recipient cells
- **Hypothesis behind this experiment:**
 - The time taken for genes to enter a donor cell is directly related to their order along the bacterial chromosome
 - The chromosome of the donor strain in an **Hfr** mating is transferred in a linear manner to the recipient strain
 - The order of genes along the chromosome can be deduced by determining the time it takes various genes to enter the recipient strain
 - Thus the interruptions of mating between **Hfr** and **F⁻** at different times would lead to various lengths of the **Hfr** chromosome being transferred to the **F⁻** recipient cell
 - If 2 bacterial cells: mated for a short period of time = only a small segment of



(a)



(b)

Figure 13.22 The Interrupted Mating Experiment. An interrupted mating experiment on $Hfr \times F^-$ conjugation. (a) The linear transfer of genes is stopped by breaking the conjugation bridge to study the sequence of gene entry into the recipient cell. (b) An example of the results obtained by an interrupted mating experiment. The gene order is *lac*-*tsx*-*gal*-*trp*.

- Hence by determining which genes were transferred during the short mating and which required longer mating times, the order of genes can be deduced
- The **Hfr** chromosome, originally circular, unwinds and is transferred to the **F**-cell in a linear fashion
- The transfer begins from specific point at the integrated **F**-factor called origin of transfer
- The farther a gene is from the origin of transfer, the later it is transferred to the **F**-
- Since different **Hfr** strains have their F plasmids inserted at different sites on the bacterial chromosome, transfer of chromosomal genes begin at different points
- In addition, the F plasmid may be inserted in either

Nutritional types of bacteria:

- Based on the requirements of nutrients bacteria are classified into,
- **Autotrophs** and **Heterotrophs**

Autotrophs

Photolithotrophic autotrophy (photolithoautotrophy)

Bacteria of this kind use light energy and have CO_2 as their carbon source. Ex. Purple and green sulfur bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen,

Chemolithotrophic autotrophy (chemolithoautotrophy)

Bacteria of this kind oxidizes reduced inorganic compounds such as iron, nitrogen or sulfur molecules to derive both energy and electrons for biosynthesis.

Ex. Sulfur-oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron-oxidizing bacteria

Heterotrophs

Photoorganotrophic heterotrophy (photo-organoheterotrophy)

Bacteria of this kind use organic matter as their electron donor and carbon source.

Ex. Purple and green sulfur bacteria

Chemoorganotrophic heterotrophy (chemo-organoheterotrophy)

Bacteria of this kind use organic compounds as sources of energy, hydrogen, electrons and carbon.

Ex. Nonphotosynthetic bacteria

- **Mixotrophy:** Bacteria of this kind combine chemolithoautotrophic and heterotrophic metabolic processes. They show great metabolic flexibility and alter their metabolic patterns in response to environmental changes

Ex. Purple nonsulfur bacteria acts as photoorganotrophic heterotrophs in the absence of oxygen but oxidize organic molecules and function simultaneously

Requirements for nitrogen, phosphorus and sulfur:

- **Nitrogen-** Needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors and other substances
 - Bacteria reduces nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction; many of bacteria can reduce and assimilate atmospheric nitrogen using the nitrogenase system
 - Ex. Phototrophic bacteria, nonphotosynthetic bacteria, cyanobacteria, symbiotic bacterium ***Rhizobium***
 - **Phosphorus-** Bacteria uses inorganic phosphate as their phosphorus source and incorporate it directly; low phosphate levels actually limit microbial growth in many aquatic environments
 - Ex. ***E. coli*** uses both organic and inorganic phosphate
 - **Sulfur-** Needed for the synthesis of substances like the amino acid Cysteine and Methionine, some carbohydrates, Biotin and Thiamine
 - Bacteria uses sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; few require a reduced form of sulfur such as Cysteine
- Other than these elements, some organic compounds required because they are essential cell components or precursors of such components and cannot be synthesized by bacteria and are called **Growth Factors**

There are 3 major classes of growth factors:

- **Amino acids-** Needed for protein synthesis
- **Purines and pyrimidines-** Needed for nucleic acid synthesis
- **Vitamins-** Small organic molecules that usually make up all or part of enzyme cofactors and only very small amounts sustain growth

Vitamin	Functions	Examples of bacteria requiring vitamin
Biotin	Carboxylation (CO ₂ fixation) one carbon metabolism	<i>Leuconostoc mesenteroides</i>
Cyanocobalamine (B ₁₂)	Molecular rearrangements one carbon metabolism- carries methyl groups	<i>Lactobacillus spp.</i>
Folic acid	One-carbon metabolism	<i>Enterococcus faecalis</i>
Lipoic acid	Transfer of acyl groups	<i>Lactobacillus casei</i>
Pantothenic acid	Precursor of coenzyme A- carries acyl groups (pyruvate oxidation, fatty acid metabolism)	<i>Proteus morganii</i>
Pyridoxine (B ₆)	Amino acid metabolism (Ex. Transamination)	<i>Lactobacillus spp.</i>
Niacin (nicotinic acid)	Precursor of NAD and NADP- carry electrons and hydrogen atoms	<i>Brucella abortus</i> <i>Haemophilus influenzae</i>
Riboflavin	Precursor of FAD and FMN- carry electrons or hydrogen atoms	<i>Caulobacter vibrioides</i>
Thiamine (B ₁)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	<i>Bacillus anthracis</i>

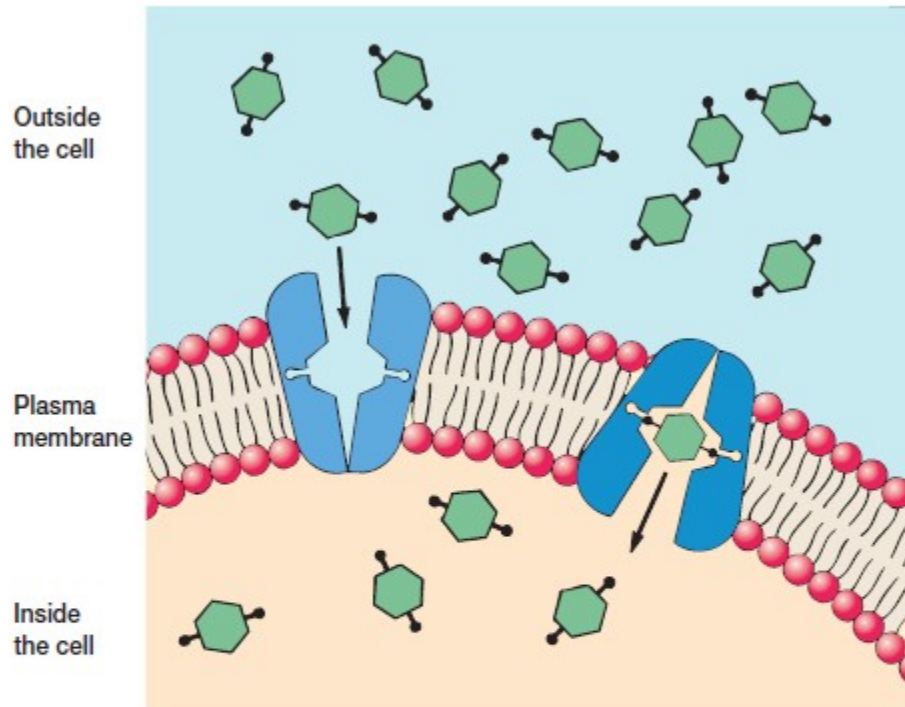


Figure 5.2 A Model of Facilitated Diffusion. The membrane carrier can change conformation after binding an external molecule and subsequently release the molecule on the cell interior. It then returns to the outward oriented position and is ready to bind another solute molecule. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside.

Uptake of nutrients:

- The transport of organic molecules while modifying them happens by the process of **Group Translocation**
- Many sugars are transported and phosphorylated simultaneously

1. Transport of molecules through **Facilitated Diffusion** (with the aid of channel proteins like **MIP** family - the transport of glycerol through facilitated diffusion with the help of **Aquaporins**)

- Ex. ***E. coli*, *Salmonella typhimurium*, *Pseudomonas*, *Bacillus*** etc.

2. Transport of solute molecules to higher concentrations, or against a concentration gradient, with the use of metabolic energy input- **Active Transport** with the aid of binding protein transport systems or **ATP-binding cassette transporters (ABC transporters)** also occur in many classes of bacteria

- Ex. *E. coli* transports a variety of sugars (Arabinose, Maltose, Galactose, Ribose) and amino acids (Glutamate, Histidine, Leucine) by this mechanism
- Some substances entering Gram-negative bacteria must pass through the outer membrane before ABC transporters and other active transport systems can take action with the help of small

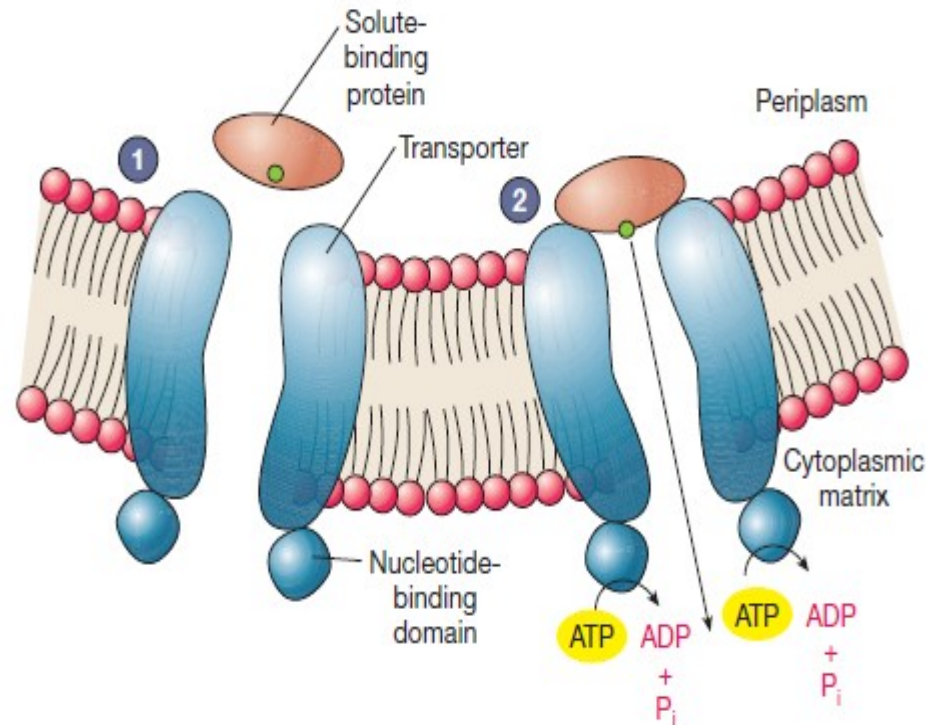
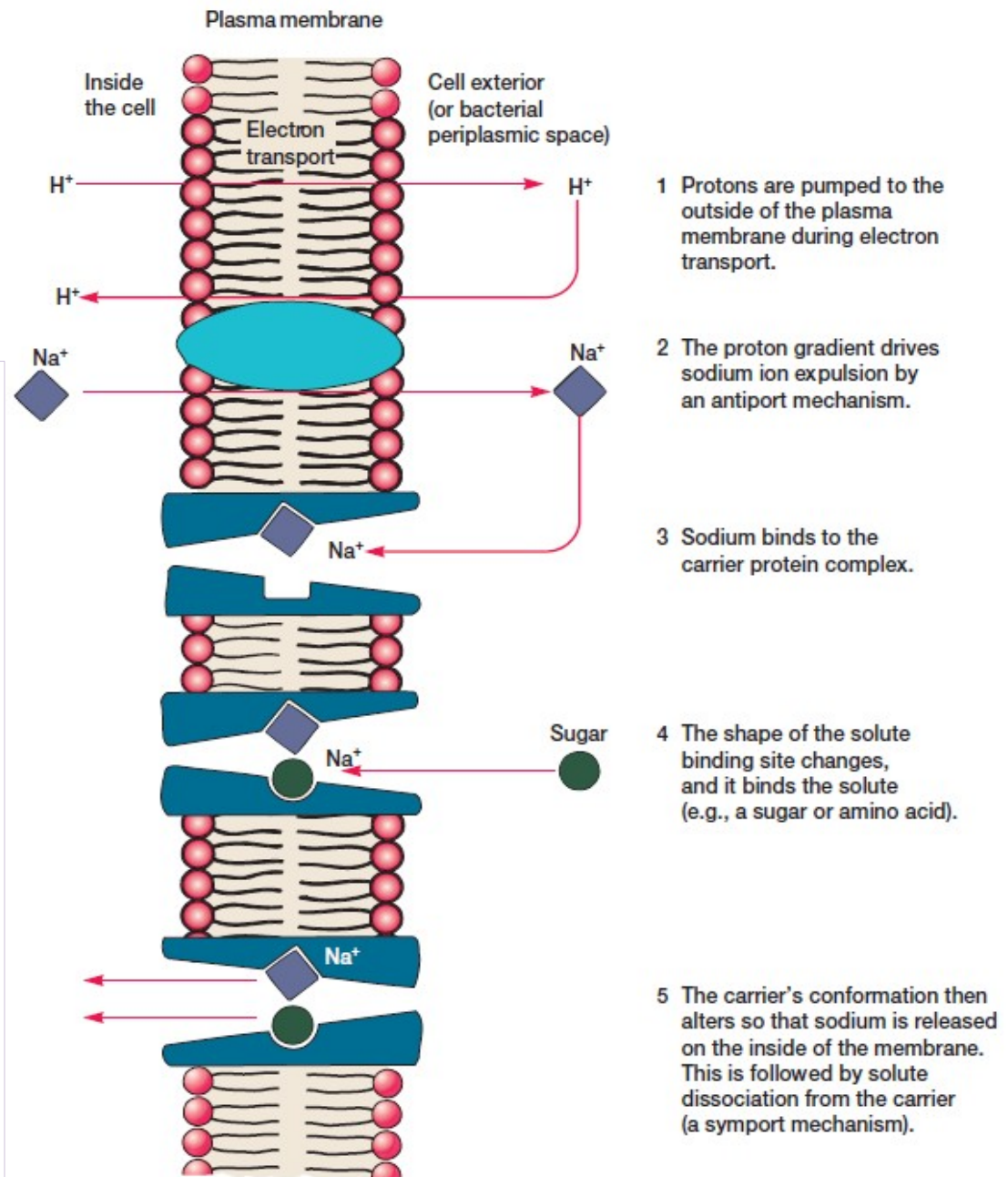


Figure 5.3 ABC Transporter Function. (1) The solute binding protein binds the substrate to be transported and approaches the ABC transporter complex. (2) The solute binding protein attaches to the transporter and releases the substrate, which is moved across the membrane with the aid of ATP hydrolysis. See text for details.

Figure 5.4 Active Transport Using Proton and Sodium Gradients. (1) Protons are pumped to the outside of the plasma membrane during electron transport. (2) The proton gradient drives sodium ion expulsion by an antiport mechanism. (3) Sodium binds to the carrier protein complex. (4) The shape of the solute binding site changes, and it binds the solute (e.g., a sugar or amino acid). (5) The carrier's conformation then alters so that sodium is released on the inside of the membrane. This is followed by solute dissociation from the carrier (a symport mechanism).



3. Bacteria also uses **Proton Gradients** generated during electron transport to drive active transport to drive active transport

Ex. Lactose permease of *E. coli*

4. Bacteria uses **Proton Symport** to take up amino acids and organic acids like Succinate and Malate

Ex. *E. coli*

5. Bacteria also transport substances through **linked transport** where the substances move in opposite direction (**Antiport**)

Ex. *E. coli* transport proteins

6. Group translocation

- A process in which molecule is transported into the cell while being chemically altered (this can be classified as a type of **energy- dependent transport** because metabolic energy is used)
- The best-known group translocation system is **phosphoenolpyruvate: sugar phosphotransferase system (PTS)**
- It transports a variety of sugars into the cells while phosphorylating them using **phosphoenolpyruvate (PEP)** as the phosphate donor
- **PEP + sugar (outside) → pyruvate + sugar - P (inside)**
- Ex. *E. coli* and *Salmonella typhimurium* (facultatively anaerobic bacteria)
- Aerobic bacteria lacks **PTSs**

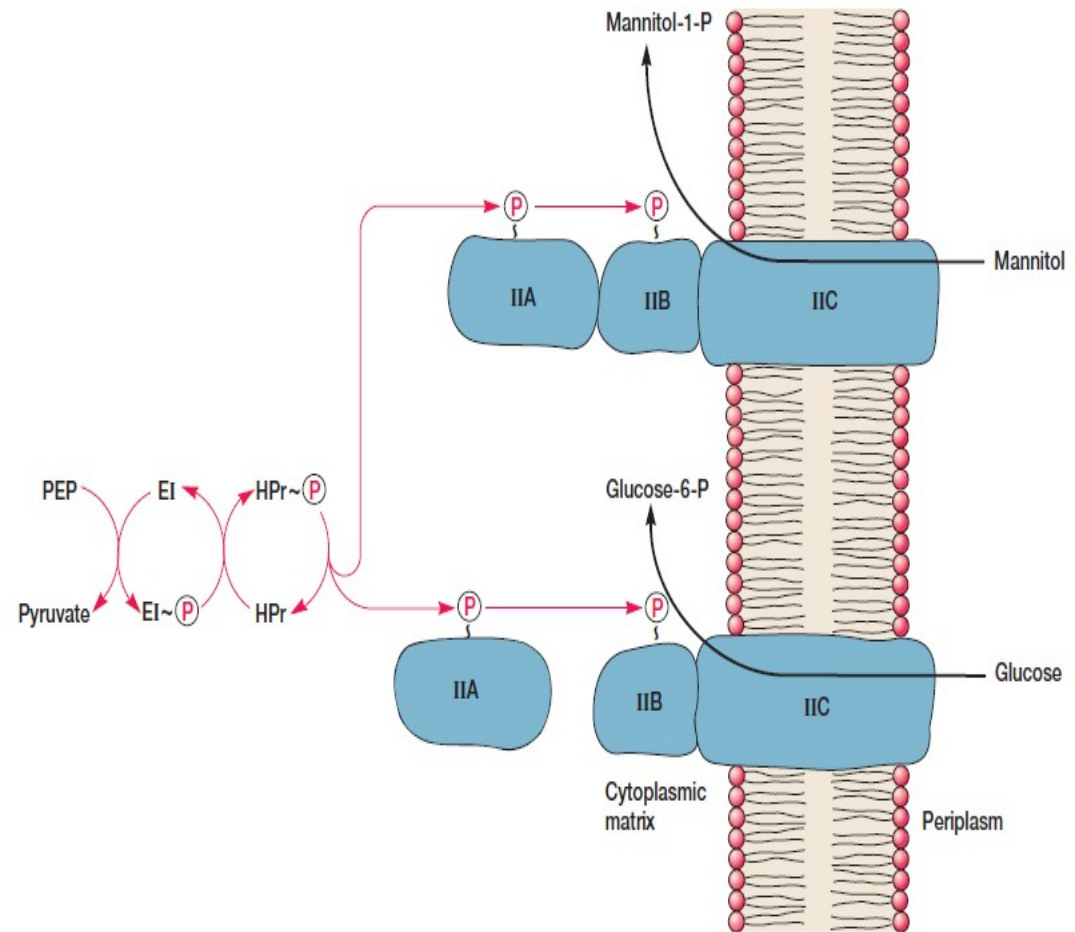
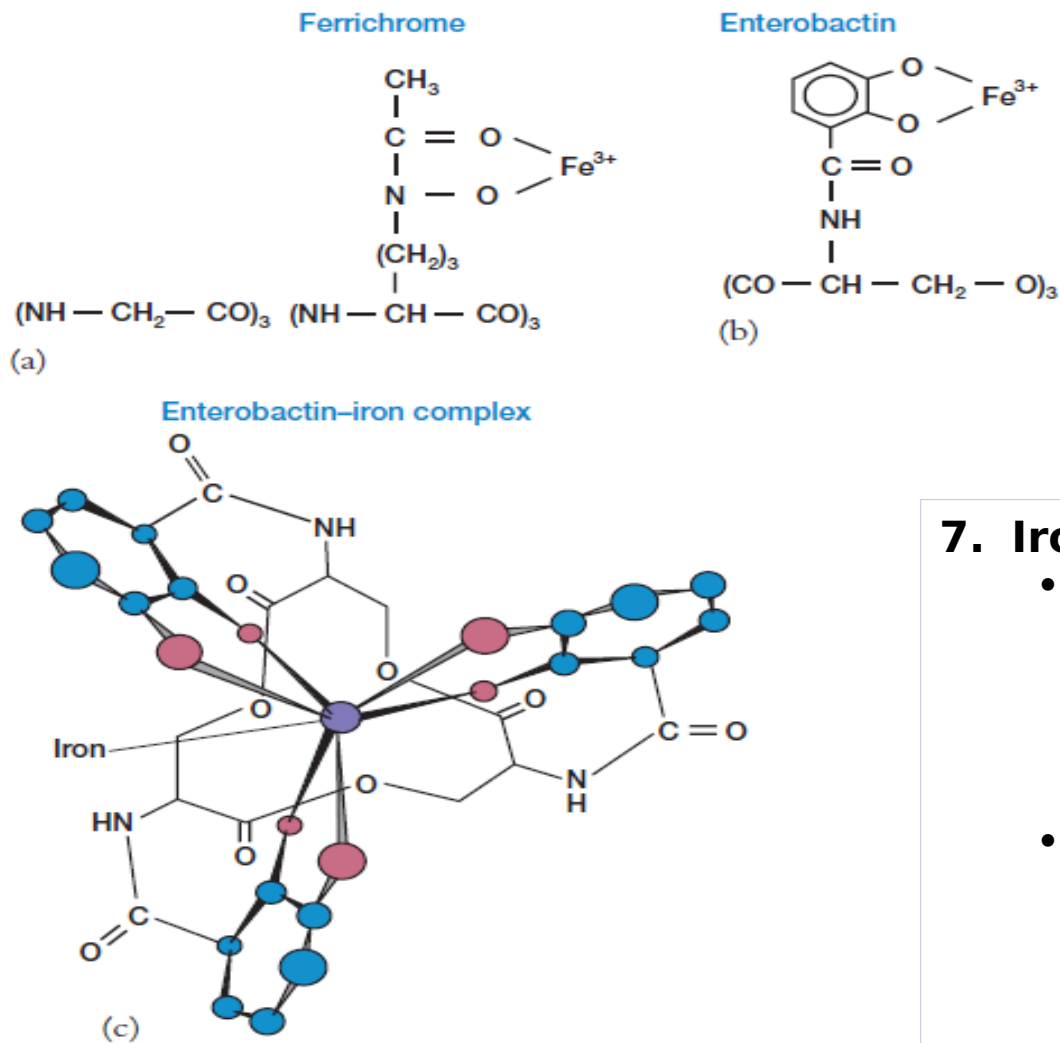


Figure 5.5 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). The high-energy phosphate is transferred from HPr to the soluble EIIA. EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system. In either case the phosphate moves from EIIA to EIIB, and then is transferred to the sugar during transport through the membrane. Other relationships between the EII components are possible. For example, IIA and IIB may form a soluble protein separate from the membrane complex; the phosphate still moves from IIA to IIB and then to the membrane domain(s).



7. Iron uptake

- Iron uptake is made difficult by the extreme insolubility of ferric iron and its derivatives, which leaves little free iron available for transport
- Many bacteria has **Siderophores** to overcome this difficulty which are low molecular weight molecules that are able to complex with ferric iron and supply it to the cells

Figure 5.6 Siderophore Ferric Iron Complexes. (a) Ferrichrome is a cyclic hydroxamate [$-\text{CO}-\text{N}(\text{O}^-)-$] molecule formed by many fungi. (b) *E. coli* produces the cyclic catecholate derivative, enterobactin. (c) Ferric iron probably complexes with three siderophore groups to form a six-coordinate, octahedral complex as shown in this illustration of the enterobactin-iron complex.

Culture media for bacterial growth:

Culture media- solid or liquid medium that contain all the nutrients required for growth

- Used to grow, transport and store microorganisms
- Employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties
- Culture media can be classified on the basis on

Physical Nature:

1. Liquid
2. Semi-solid
3. Solid

Chemical nature:

4. Synthetic
5. Complex

Functional type:

6. Supportive
7. Enriched
8. Selective
9. Differential



Minimal media

- Contain minimum nutrients possible for growth of wild type microorganisms
- Contain- carbon source (glucose)+ inorganic salts+ water

Supplementary media

- Type of minimal media
- Contains single selected agent
- Contains amino acid or sugar for culturing of specific auxotroph

Synthetic media

- Chemically-defined medium
- Exact composition is known

Complex media

- Undefined medium
- Exact composition is not known
- Ex. Nutrient broth, tryptic soy broth, MacConkey agar

Enriched media

- Contains component that permits the growth of specific types or species of bacteria
- Ex. Blood agar

Selective media

- Contains component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species
- Ex. Bile salts and crystal violet favor growth of Gram-Positive bacteria by inhibiting the growth of Gram-Negative bacteria
- Ex. Eosin methylene blue agar, MacConkey agar, Mannitol salt

Differential media

- Media which allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the media
- Ex. Blood agar (distinguishes between Hemolytic and Non-Hemolytic bacteria)

microbial population grown from a single cell

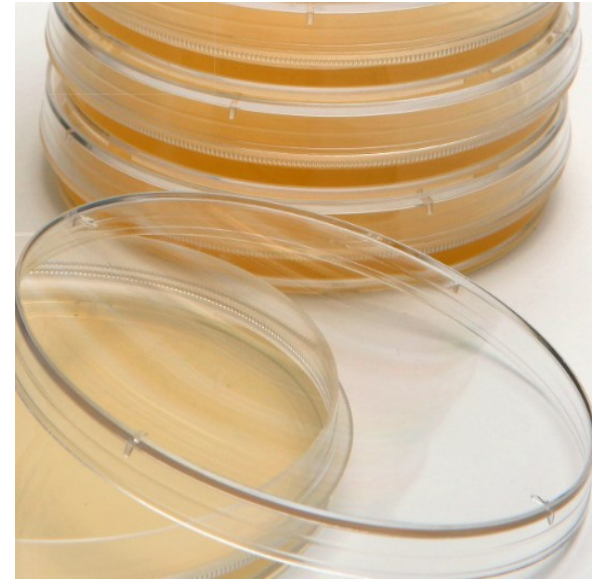
- Usually derived from mixed culture



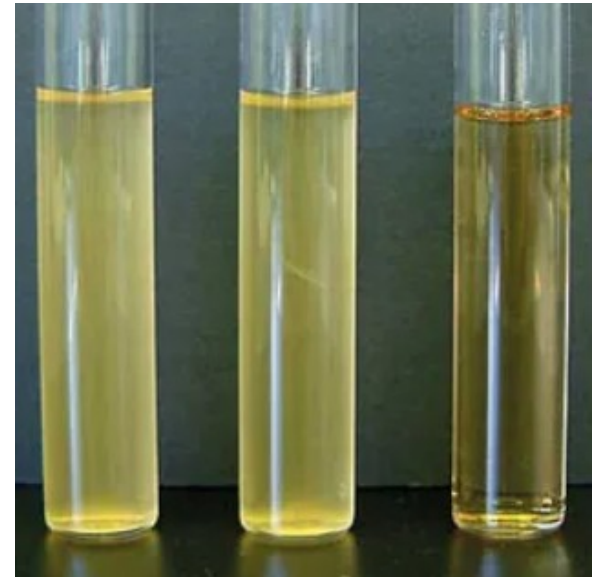
Minimal Agar



Blood Agar



M9 Agar Plate Media



Mannitol Salt Agar Medium Methylen Blue Agar Tryptic Soy Broth

Bacteria growth:

- An orderly increase in the quantity of cellular constituents (cell mass) and number
- Depends upon the ability of the cell to form new protoplasm from nutrients available in the environment
- The increase in bacterial cell number occurs by cell division= **Binary Fission**

- **Binary fission:**

- Begins when DNA of the cell is replication
 - Each circular strand of DNA attaches to the plasma membrane
 - The cell elongates, causing the two replicated DNA to separate
 - The plasma membrane then invaginates (grows inward) and splits the cell into two daughter cells through a process called **cytokinesis**
 - Few bacterial species reproduce by **Budding**- they form a small bud that enlarges until its size approaches that of the parent cell, and then it separates
- **FtsZ** protein (homologous to tubulin protein) plays important role in cell division
- The cellular concentration of **FtsZ** regulates the frequency of division
- At the time of septal invagination, the **FtsZ** protein is recruited from

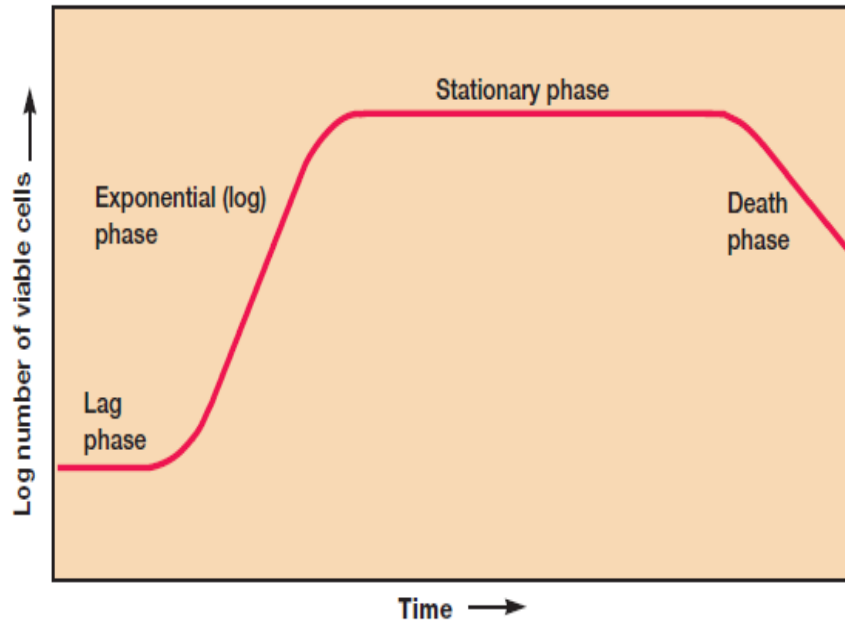


Figure 6.1 Microbial Growth Curve in a Closed System. The four phases of the growth curve are identified on the curve and discussed in the text.

Growth in batch culture can be divided into 4 distinct phases:

1. **Lag phase**
2. **Log phase**
3. **Stationary phase**
4. **Death phase**

Bacterial cell is inoculated into a flask containing fresh culture medium and incubated

The bacterial cell enters into a rapid
Growth Phase

Bacterial cells divide and increases its population in the flask medium

The increasing population of bacterial cells, after sometime, enters into a
Stationary Phase with the exhaustion of the required nutrients

Accumulation of inhibitory end products in the medium

The stationary phase of bacterial population culminates into **Death Phase** when a viable bacterial cells begins to die
A batch culture can be considered to be a closed system

Lag phase:

- It is the period of adaptation of cells to a new environment
- There is no increase in cell number but there is increase in mass
- The bacterial cells are not dormant

Log phase:

- Length of the lag phase is determined in part by the characteristics of the bacterial species and in part by conditions in the media
- Period of increase in cell number exponentially with time

Also known as **Exponential Phase**

- Rate of increase of cells in the culture is proportional to the number of cells present at any particular time
- Number of cells increase in the geometric progression
- Cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation

Stationary phase:

- The rate of exponential growth of the bacterial culture is expressed as generation time, also the doubling time of the bacterial population
- Period where the cell growth rate has leveled off and became constant
- The number of cells multiplying equals the number of cells dying
- Population growth is limited by one of three factors:
 1. Exhaustion of available nutrients
 2. Accumulation of inhibitory metabolites or end products

Death phase:

- Exhaustion of space, in this case called a lack of biological space
- Period in which the viable cell population declines
- The number of viable cells decreases geometrically (exponentially) which essentially reverses the growth of bacterial cells

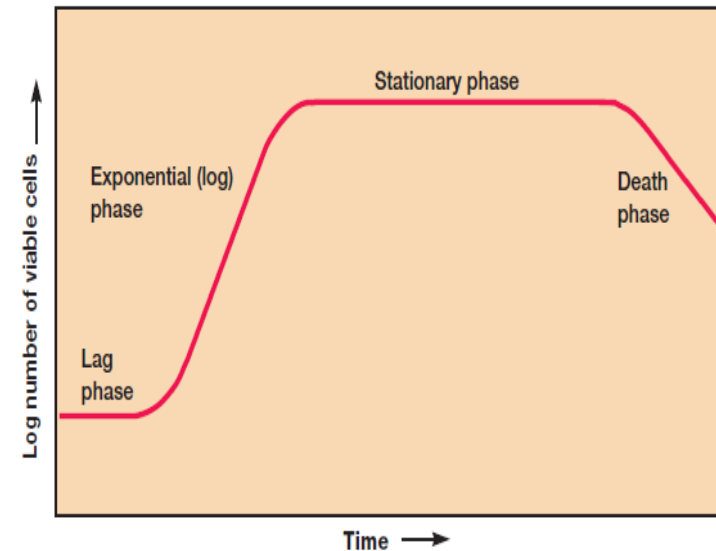


Figure 6.1 Microbial Growth Curve in a Closed System. The four phases of the growth curve are identified on the curve and discussed in the text.

Calculation of generation time:

- The interval for the formation of two cells from one is called generation and the time required for this to occur is called generation time
- Generation time = $\frac{t \text{ (time in minutes or hours)}}{n \text{ (number of generations)}}$

Calculation of number of generation

- If N^0 = number of bacteria at the beginning of a time interval
- N = number of bacteria at the end of time interval
- n = number of generations (number of times the cell population doubles during the time interval)
- $N = N^0 \times 2^n$
- This becomes
- $\log N = \log N^0 + n \log 2$
- The number of generations (n)

$$n = \frac{\log N - \log N^0}{\log 2} = \frac{\log N - \log N^0}{0.301} = 3.3 \log N / N^0$$

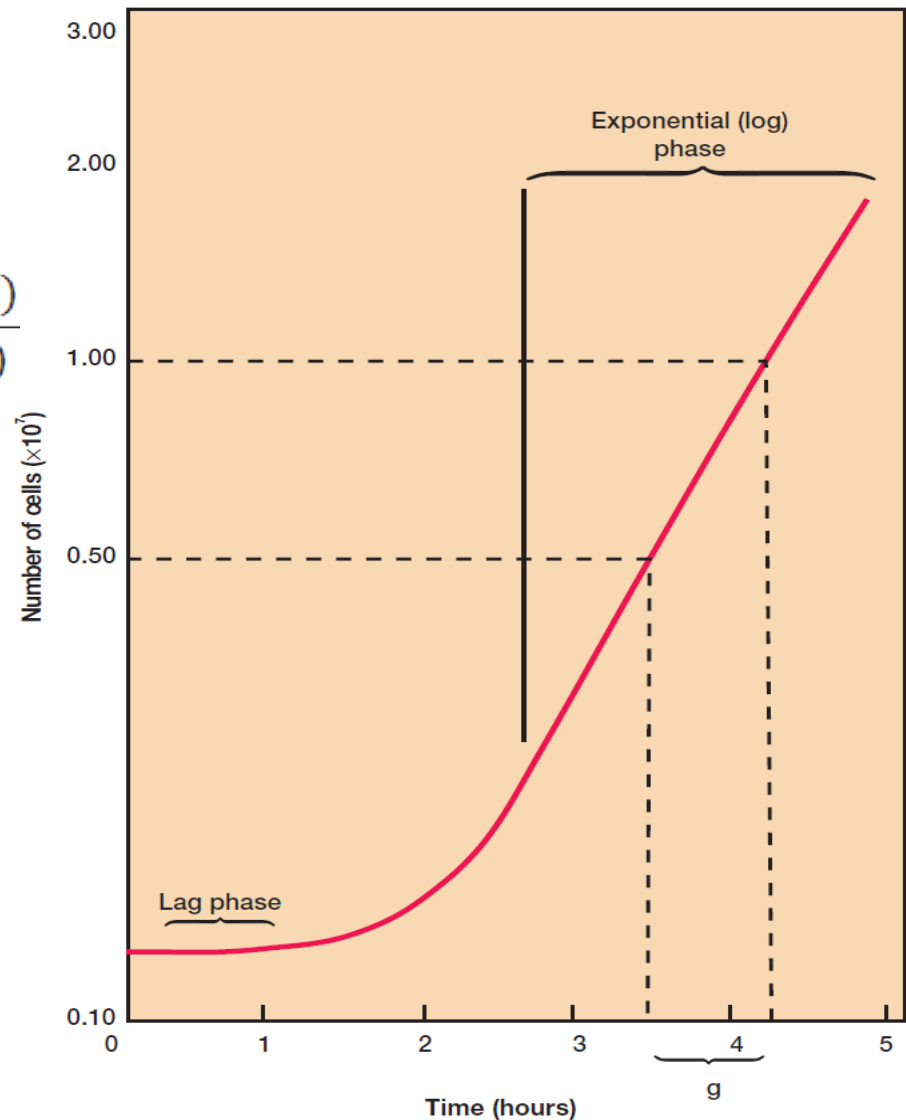


Figure 6.4 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

Calculation of bacterial cell density:

- **Cell density**= number of cells per unit volume
- **Direct measurement of cell density** -By measuring colony number of counting the number of cell in known volume with a microscope
- **Indirect method of determination of cell density**- By measuring of optical absorbance through spectrophotometer

Measurement of colony number:

- Bacterial cells form colonies on agar medium
- As bacteria divides, the progeny bacteria remains adjacent to the original bacterium
- As the number of progeny increases to about 10^6 cells, a visible cluster of bacteria appears
- This cluster is a population of bacterial cells is called a **Bacterial Colony**
- By counting the number of colonies formed when a known volume if diluted culture is plated one can determine the number of bacteria in a undiluted culture
- Usually bacterial culture contains very high number of cells, hence it must be diluted before plating, and the dilution factor must be taken into account when calculating the cell density in undiluted culture
- It can be calculated by using formula,

$$\frac{(\text{number of colonies formed})}{(\text{ml plated})} \times (\text{dilution before plating}) = \frac{\text{number of viable cells}}{\text{ml of undiluted culture}}$$

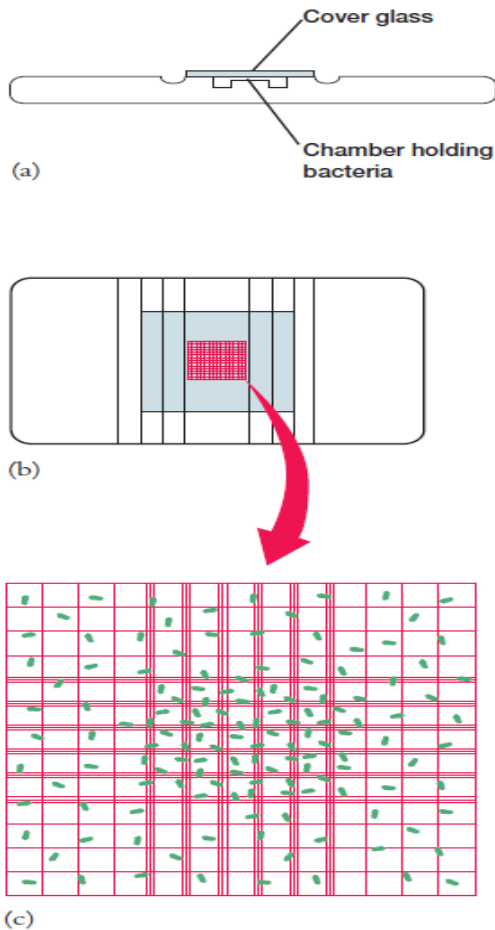


Figure 6.5 The Petroff-Hausser Counting Chamber. (a) Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension. (b) A top view of the chamber. The grid is located in the center of the slide. (c) An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at $\times 400$ to $\times 500$ magnification. The average number of bacteria in these squares is used to calculate the concentration of cells in the original sample. Since there are 25 squares covering an area of 1 mm^2 , the total number of bacteria in 1 mm^2 of the chamber is (number/square)(25 squares). The chamber is 0.02 mm deep and therefore,

$$\text{bacteria/mm}^3 = (\text{bacteria/square})(25 \text{ squares})(50).$$

The number of bacteria per cm^3 is 10^3 times this value. For example, suppose the average count per square is 28 bacteria:

$$\text{bacteria/cm}^3 = (28 \text{ bacteria}) (25 \text{ squares})(50)(10^3) = 3.5 \times 10^7.$$

Measurement of bacterial growth:

1. Direct counting: It is the method of counting the bacteria cells using a counting chamber. It also gives information about the size and morphology of bacteria.

Petroff-Hausser counting chambers can be used for counting prokaryotes; hemocytometers can be used for both prokaryotes and eukaryotes.

Prokaryotes are more easily counted in these chambers if they are stained, or when a phase-contrast or a fluorescence microscope is employed

These specially designed slides have chambers of known depth with an etched grid on the chamber bottom

The number of microorganisms in a sample can be calculated by taking into account the chamber's volume and any sample dilutions required

Disadvantages:

The microbial population must be fairly large for accuracy because such a small volume is sampled. It is also difficult to

2. Membrane filter technique: Method used to determine bacterial numbers of colonies growing on special membrane filters having pores small enough to trap bacteria

The filter is then placed on an agar medium or on a pad soaked with liquid media and incubated until each cell forms a separate colony. A colony count gives the number of bacteria in the filtered sample, and special media can be used to select for specific microorganisms

This technique is especially useful in analyzing aquatic samples

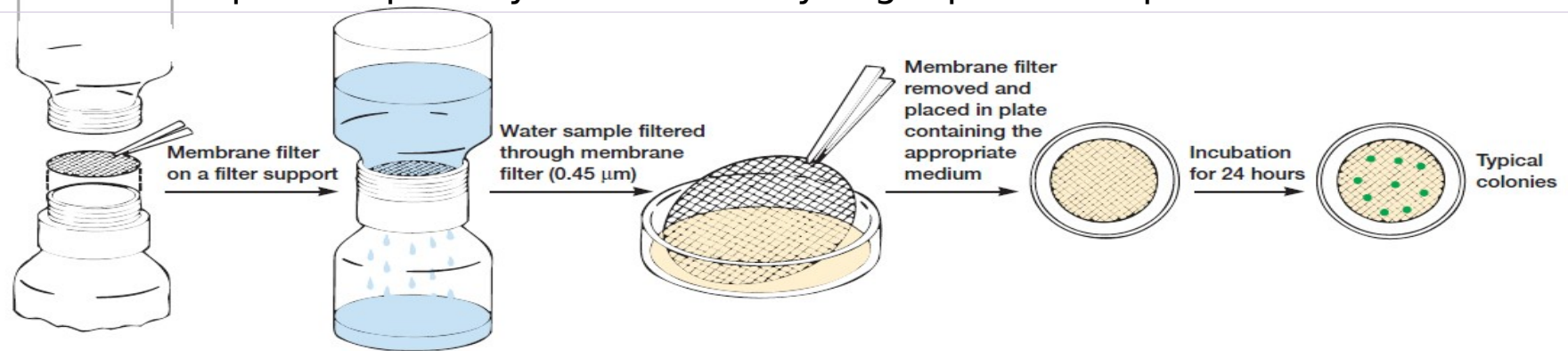


Figure 6.6 The Membrane Filtration Procedure. Membranes with different pore sizes are used to trap different microorganisms. Incubation times for membranes also vary with the medium and microorganism.

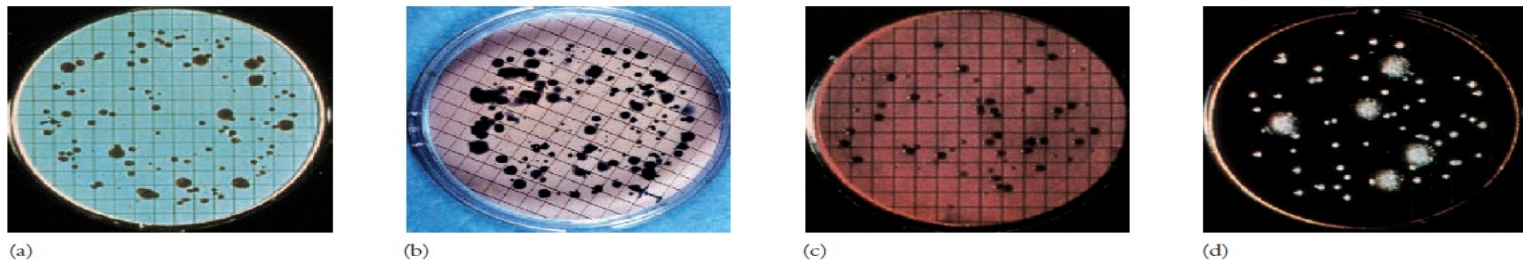
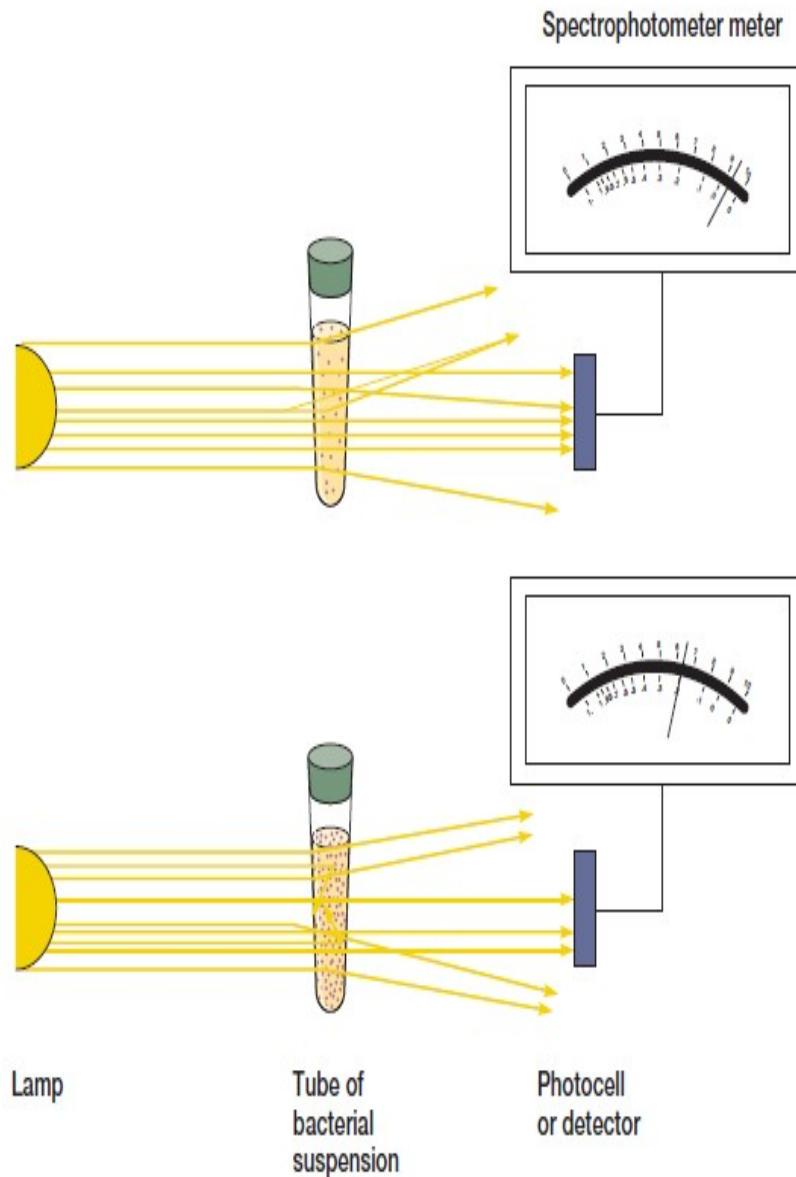


Figure 6.7 Colonies on Membrane Filters. Membrane-filtered samples grown on a variety of media. (a) Standard nutrient media for a total bacterial count. An indicator colors colonies red for easy counting. (b) Fecal coliform medium for detecting fecal coliforms that form blue colonies. (c) m-Endo agar for detecting *E. coli* and other coliforms that produce colonies with a green sheen. (d) Wort agar for the culture of yeasts and molds.



3. Measurement of cell mass: It is the techniques for measuring changes in cell mass

Principle: Microbial cells in a population are of roughly constant size, the amount of scattering is directly proportional to the biomass of cells present & indirectly related to cell number. When the concentration of bacteria reaches about 10 million cells (10^7) /ml, the medium appears slightly cloudy or turbid. Further increases in concentration result in greater turbidity and less light is transmitted through the medium. The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels

Thus population growth can be easily measured spectrophotometrically as long as the population is high enough to give detectable turbidity.

Figure 6.8 Turbidity and Microbial Mass Measurement
Determination of microbial mass by measurement of light absorption. As the population and turbidity increase, more light is scattered and the absorbance reading given by the spectrophotometer increases. The spectrophotometer meter has two scales. The bottom scale displays absorbance and the top scale, percent transmittance. Absorbance increases as percent transmittance decreases.



Influence of environmental factors on growth:

Temperature, pH, Water Availability, Oxygen plays important role in bacterial growth.

- pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity)
- **Acidophiles** have their growth optimum between pH 0 and 5.5
- **Neutrophiles**, between pH 5.5 and 8.0
- **Alkalophiles** prefer the pH range of 8.5 to 11.5
- Extreme Alkalophiles have growth optima at pH 10 or higher
- Most bacteria and protozoa are Neutrophiles

Figure 6.11 The pH Scale. The pH scale and examples of substances with different pH values. The microorganisms are placed at their growth optima.

2. Temperature

- Environmental temperature profoundly affects microorganisms
- Microbial cell temperature directly reflects that of the cell's surroundings
- The most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme catalyzed reactions
- At low temperatures a temperature rise increases the growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature
- High temperatures damage microorganisms by denaturing enzymes, transport carriers and other proteins. Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates. Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot

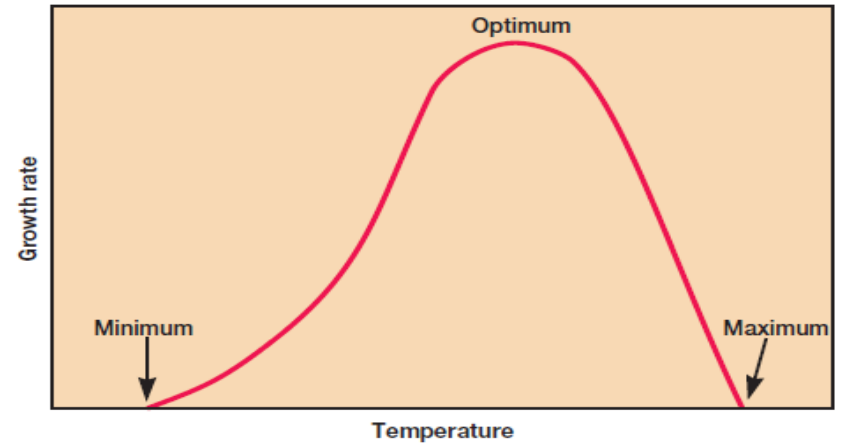


Figure 6.12 Temperature and Growth. The effect of temperature on growth rate.

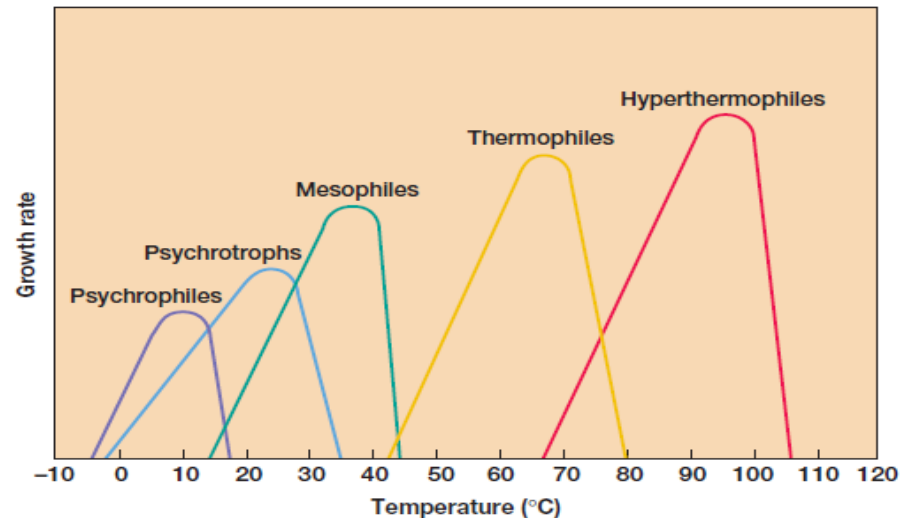


Figure 6.13 Temperature Ranges for Microbial Growth. Microorganisms can be placed in different classes based on their temperature ranges for growth. They are ranked in order of increasing growth temperature range as psychrophiles, psychrotrophs, mesophiles, thermophiles, and hyperthermophiles. Representative ranges and optima for these five types are illustrated here.

Table 6.5 Temperature Ranges for Microbial Growth

Microorganism	Cardinal Temperatures (°C)		
	Minimum	Optimum	Maximum
Nonphotosynthetic Procaryotes			
<i>Bacillus psychrophilus</i>	−10	23–24	28–30
<i>Micrococcus cryophilus</i>	−4	10	24
<i>Pseudomonas fluorescens</i>	4	25–30	40
<i>Staphylococcus aureus</i>	6.5	30–37	46
<i>Enterococcus faecalis</i>	0	37	44
<i>Escherichia coli</i>	10	37	45
<i>Neisseria gonorrhoeae</i>	30	35–36	38
<i>Thermoplasma acidophilum</i>	45	59	62
<i>Bacillus stearothermophilus</i>	30	60–65	75
<i>Thermus aquaticus</i>	40	70–72	79
<i>Sulfolobus acidocaldarius</i>	60	80	85
<i>Pyrococcus abyssi</i>	67	96	102
<i>Pyrodictium occultum</i>	82	105	110
<i>Pyrolobus fumarii</i>	90	106	113
Photosynthetic Bacteria			
<i>Rhodospirillum rubrum</i>	ND ^a	30–35	ND
<i>Anabaena variabilis</i>	ND	35	ND
<i>Oscillatoria tenuis</i>	ND	ND	45–47
<i>Synechococcus eximius</i>	70	79	84

3. Oxygen

- An organism able to grow in the presence of atmospheric O_2 is an **Aerobe**, whereas one that can grow in its absence is an **Anaerobe**
- Oxygen serves as the terminal electron acceptor for the electron- transport chain in aerobic respiration.
- **Facultative anaerobes** do not require O_2 for growth but do grow better in its presence. In the presence of oxygen they will use aerobic respiration
- **Aerotolerant anaerobes** simply ignore O_2 and grow equally well whether it is present or not
- **Strict or obligate anaerobes** do not tolerate O_2 at all and die in its presence
- **Aerotolerant** and strict anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for this purpose.
- **Microaerophiles** are damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels below the range of 2 to 10% for growth.

Figure 6.14 Oxygen and Bacterial Growth. An illustration of the growth of bacteria with varying responses to oxygen. Each dot represents an individual bacterial colony within the agar or on its surface. The surface, which is directly exposed to atmospheric oxygen, will be aerobic. The oxygen content of the medium decreases with depth until the medium becomes anaerobic toward the bottom of the tube. The presence and absence of the enzymes superoxide dismutase (SOD) and catalase for each type are shown.

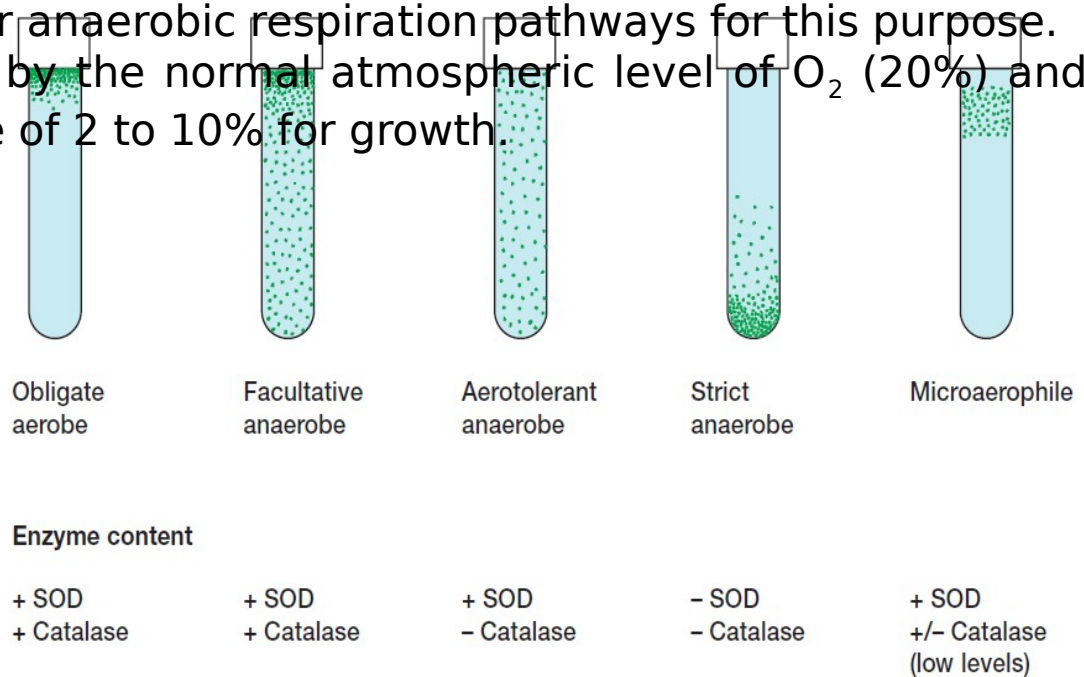


Table 6.3 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	<i>Staphylococcus aureus</i> , <i>Saccharomyces rouxii</i>
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	<i>Halobacterium</i> , <i>Dunaliella</i> , <i>Ectothiorhodospira</i>
pH		
Acidophile	Growth optimum between pH 0 and 5.5	<i>Sulfolobus</i> , <i>Picrophilus</i> , <i>Ferroplasma</i> , <i>Acontium</i> , <i>Cyanidium caldarium</i>
Neutrophile	Growth optimum between pH 5.5 and 8.0	<i>Escherichia</i> , <i>Euglena</i> , <i>Paramecium</i>
Alkalophile	Growth optimum between pH 8.5 and 11.5	<i>Bacillus alcalophilus</i> , <i>Natronobacterium</i>
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	<i>Bacillus psychrophilus</i> , <i>Chlamydomonas nivalis</i>
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	<i>Listeria monocytogenes</i> , <i>Pseudomonas fluorescens</i>
Mesophile	Has growth optimum around 20–45°C	<i>Escherichia coli</i> , <i>Neisseria gonorrhoeae</i> , <i>Trichomonas vaginalis</i>
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	<i>Bacillus stearothermophilus</i> , <i>Thermus aquaticus</i> , <i>Cyanidium caldarium</i> , <i>Chaetomium thermophile</i>
Hyperthermophile	Has an optimum between 80 and about 113°C	<i>Sulfolobus</i> , <i>Pyrococcus</i> , <i>Pyrodictium</i>
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O ₂ for growth.	<i>Micrococcus luteus</i> , <i>Pseudomonas</i> , <i>Mycobacterium</i> ; most algae, fungi, and protozoa
Facultative anaerobe	Does not require O ₂ for growth, but grows better in its presence.	<i>Escherichia</i> , <i>Enterococcus</i> , <i>Saccharomyces cerevisiae</i>
Aerotolerant anaerobe	Grows equally well in presence or absence of O ₂	<i>Streptococcus pyogenes</i>
Obligate anaerobe	Does not tolerate O ₂ and dies in its presence.	<i>Clostridium</i> , <i>Bacteroides</i> , <i>Methanobacterium</i> , <i>Trepomonas agilis</i>
Microaerophile	Requires O ₂ levels below 2–10% for growth and is damaged by atmospheric O ₂ (20%).	<i>Campylobacter</i> , <i>Spirillum volutans</i> , <i>Treponema pallidum</i>
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures.	<i>Photobacterium profundum</i> , <i>Shewanella benthica</i> , <i>Methanococcus jannaschii</i>

Control of microbial growth:

- To inhibit or prevent growth of microorganisms
- Control is affected in two basic ways:
 1. By killing microorganisms
 2. By inhibiting the microorganism
- Control of growth involves the use of physical or chemical agents which either kill or prevent the growth of microorganisms
 - Agents which kills cells are called **cidal agents**
 - Agents which inhibit the growth of cells are called **static agents**
- **Bactericidal**- killing bacteria; **Bacteriostatic**- inhibiting the growth of bacterial cells

Microbial control methods:**1. Physical agents:**

- Heat
- Sterilization
- Tyndallization
- Pasteurization
- Filtration
- Radiations

2. Chemical agents:

- Disinfectants
- Antiseptics
- Antibiotics

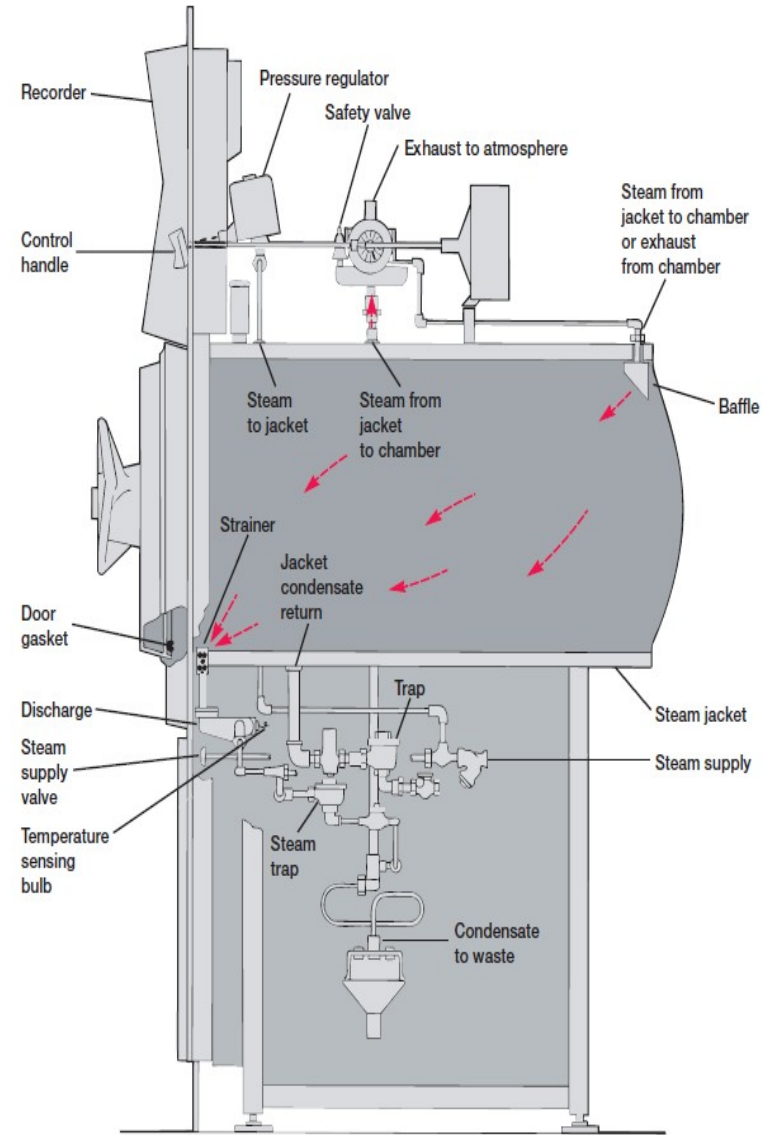
Microbial control methods:

Physical agents:

1. **Heat-** Most rapid and efficient physical agent used to control a population of microbes
 - Both dry and moist heat is used to kill microbes
 - Moist heat generally coagulates and causes denaturation in microorganisms
 - In dry heat-oxidizes the large molecules



(a)



(b)

Figure 7.3 The Autoclave or Steam Sterilizer. (a) A modern, automatically controlled autoclave or sterilizer.

(b) Longitudinal cross section of a typical autoclave showing some of its parts and the pathway of steam. (b) From John J. Perkins, *Principles and Methods of Sterilization in Health Science*, 2nd edition, 1969. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

Sterilization:

- Complete and total disruption or elimination of all viable organisms including microbial endospore in or on a substance being sterilized
- Its procedures involve the use of heat, radiation or chemical or physical removal of cells
- Common process used for sterilization:
 - Flaming
 - Autoclaving (steam under pressure)
 - Tyndallization (steam not under pressure)

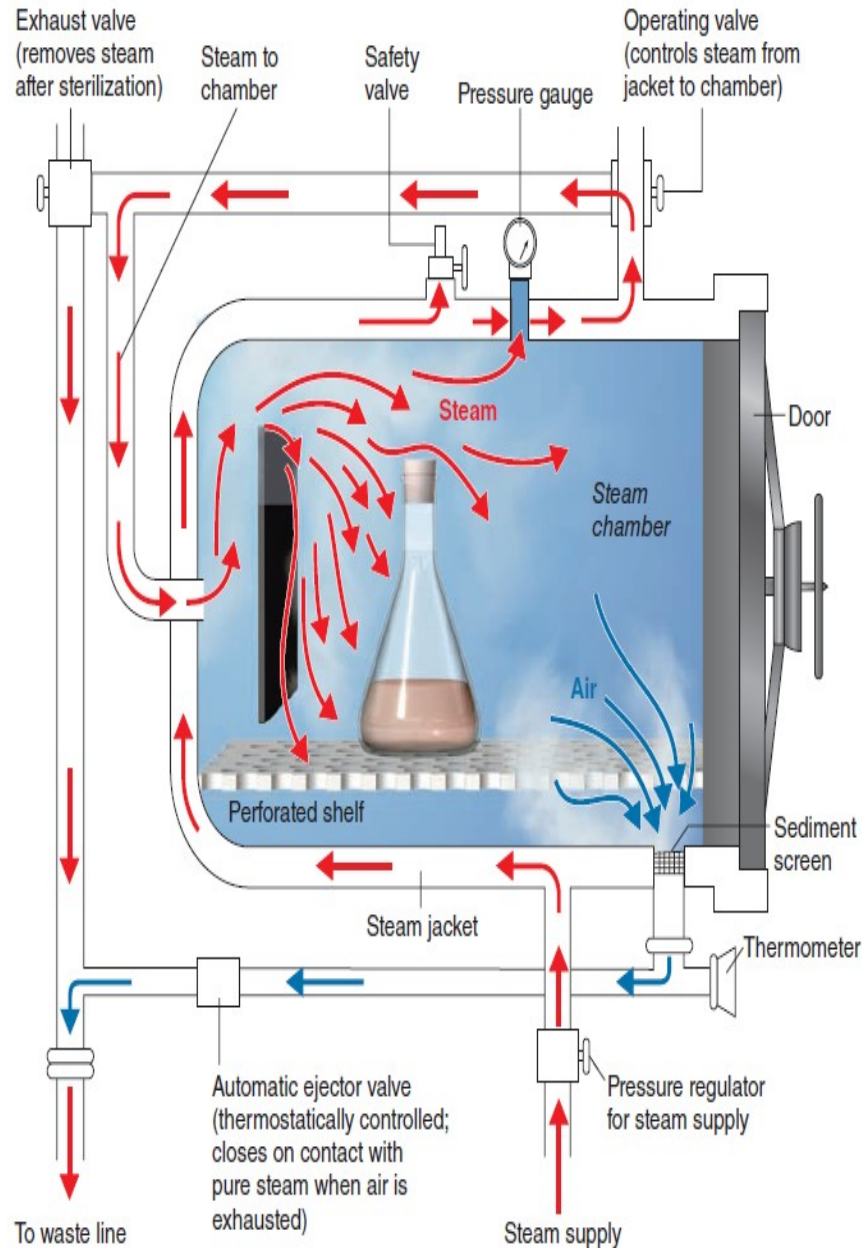


Figure 7.2 An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Q How would an empty, uncapped flask be positioned for sterilization in an autoclave?

Tyndallization: The process used to sterilize culture media that might be spoiled by exposure at higher temperature

- This heat sensitive media are steamed for 30-45 minutes each time for 3 successive days
- On the 1st occasion vegetative bacteria are killed; any spores that survive will germinate in the nutrient medium overnight producing vegetative form that are

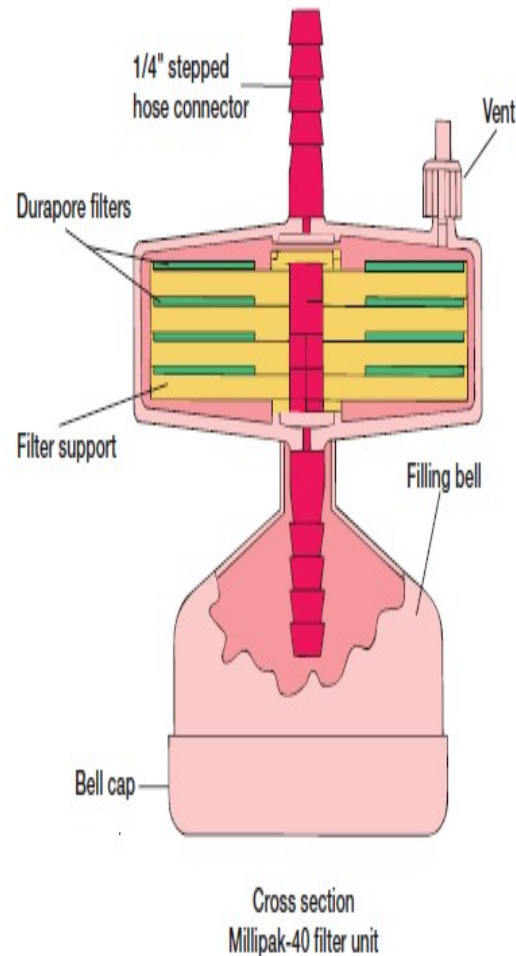


Pasteurization:

- Brief heat treatment
- Used to reduce the number of spoilage organisms and to kill disease causing microbes
- It uses precisely controlled heat to reduce the microbial populations in milk and other heat sensitive liquids
- This process is named after Louis Pasteur (first used heat for controlling the spoilage of the wine)
- This treatment does not kill all organisms and is therefore not synonymous with sterilization
- Milk is usually pasteurized by heating, typically at 63°C for 30 minutes (batch

3. Filtration- Physical removal of all cells in a liquid or gas

- Important to sterilize solutions which would be denatured by heat (Ex. Antibiotics, amino acids, vitamins etc.)
- In this process solutions or gases are passed through a filter of sufficient pore diameter (generally 0.22 micron) to remove the smallest known bacterial cells
- It is commonly carried out using membrane filters (made from cellulose esters like cellulose acetate, cellulose nitrate, collodion etc)
- Range of pore sizes are available
- Bacterial filters have a



(a)



(b)

Figure 7.4 Membrane Filter Sterilization. A membrane filter outfit for sterilizing medium volumes of solution. (a) Cross section of the membrane filtering unit. Several membranes are used to increase capacity. (b) A complete filtering setup. The solution to be sterilized is kept in the Erlenmeyer flask, 1, and forced through the filter by a peristaltic pump, 2. The solution is sterilized by flowing through a membrane filter unit, 3, and into a sterile container. A wide variety of other kinds of filtering outfits are also available.

2. Radiations- Has antimicrobial effects

- Ex. X- rays, gamma rays form free radicals in the cytoplasm and the free radicals damage microbial protein and DNA
- Ultraviolet radiations damages nucleic acids

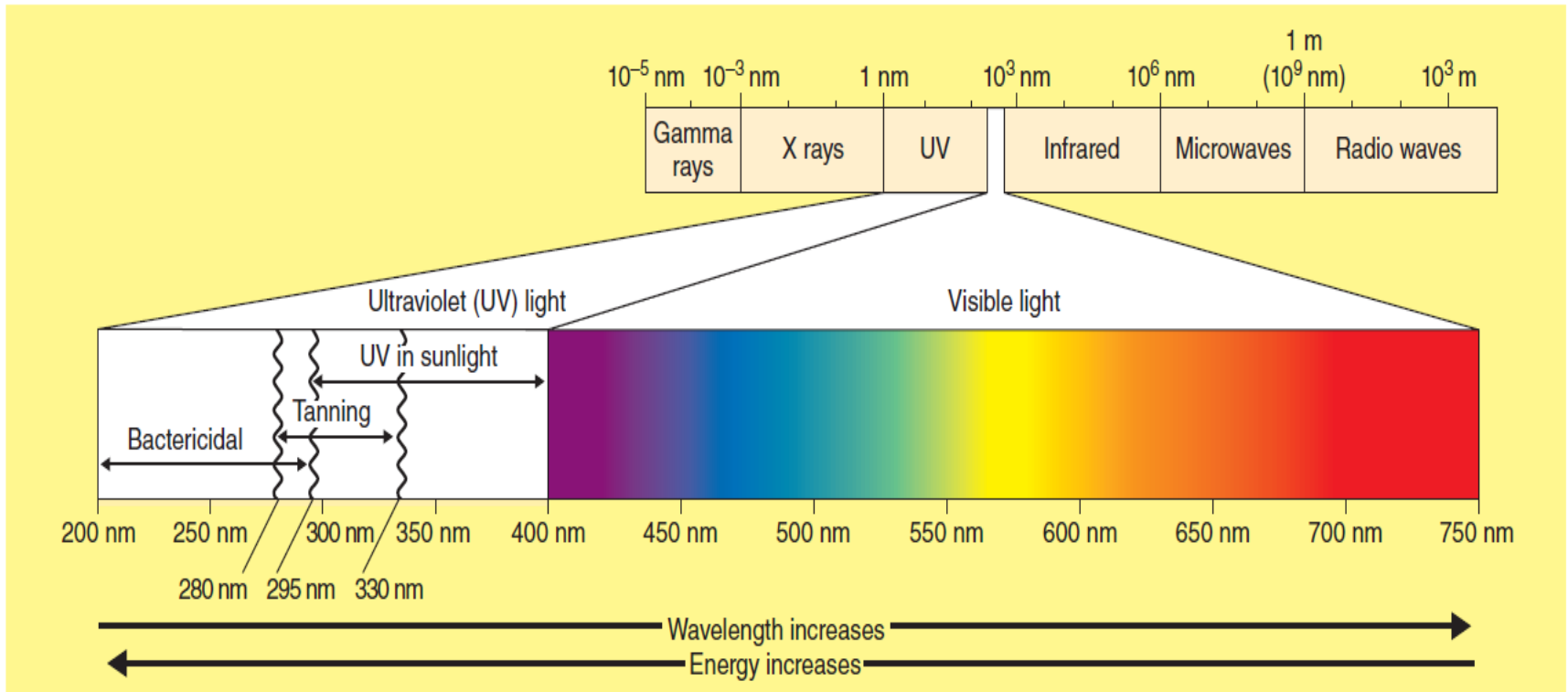


Figure 7.5 The radiant energy spectrum. Visible light and other forms of radiant energy radiate through space as waves of various lengths. Ionizing radiation, such as gamma rays and X rays, has a wavelength shorter than 1 nm. Nonionizing radiation, such as ultraviolet (UV) light, has a wavelength between 1 nm and about 380 nm, where the visible spectrum begins.

Chemical agents:

- Chemicals like alcohol, aldehyde, heavy metals, halogens and dyes are used as antimicrobial agents
- Alcohol (**ethyl alcohol** and **isopropyl alcohol**)- denatures proteins and are used as an antiseptic
- 2 heavy metals- **Mercury** and **Silver**- used as an antimicrobial agents
 - **Mercury**- used as **Mercuric Chloride** and as a component of mercurochrome
 - **Silver**- used as **Silver Nitrate**
- 2 halogens- **Chlorine** and **Iodine**- acts as oxidizing agents
 - Reacts with the amino acids in proteins and change the nature of proteins

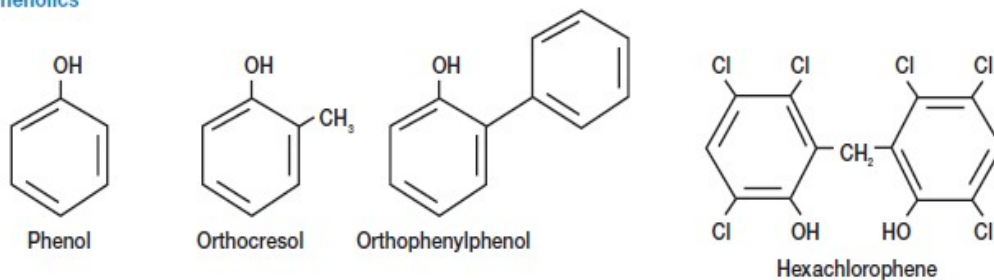
Disinfectants:

- Gases- **Ethylene Oxide**, **Formaldehyde** and **Beta Propiolactone**- used for sterilization
- Chemical that kills microorganisms
- Used on inanimate objects
- Ex. **Hypochlorites**, **Chlorine Compounds**, **Copper Sulfate**, **Formaldehyde** (reacts with NH_2 , SH and COOH groups)
- **Phenolics compounds** (Denature proteins and disrupts cell membranes) and **Mercuric Chloride** (inactivates proteins by reacting with sulfide groups)

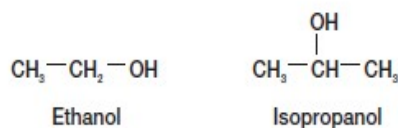
Antiseptics:

- Chemical agents that kills or inhibits growth of microorganisms and are non toxic enough to be used to living tissues
- **Ethanol**- denatures proteins and solubilizes lipids
- **Silver Nitrate**- precipitates proteins **Iodine solution**- inactivates proteins
- **Detergents** -disrupts cell

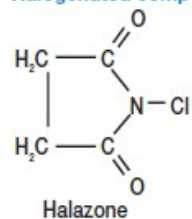
Phenolics



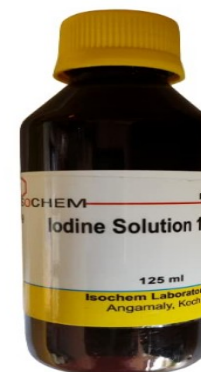
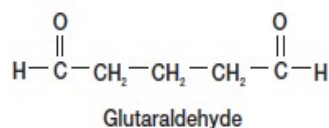
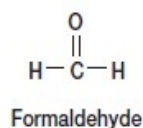
Alcohols



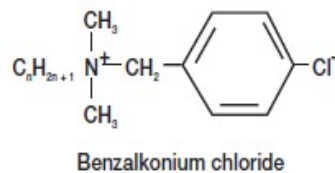
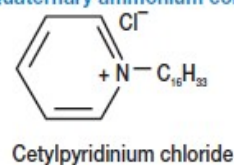
Halogenated compound



Aldehydes



Quaternary ammonium compounds



Gases

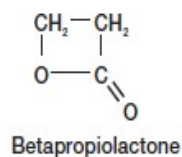
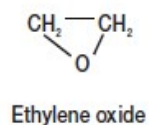


Figure 7.7 Disinfectants and Antiseptics. The structures of some frequently used disinfectants and antiseptics.

Antibiotics:

- Low molecular weight antimicrobial agents produces as a secondary metabolites by microorganisms that kill or inhibit other microorganisms
- Chemical of natural origin which can kill or inhibit the growth of other types of cells
- These has cidal effect or a static effect on a range of microbes
- Range of bacteria or other microorganisms that are affected by a certain antibiotic is expressed as its spectrum of action
- Antibiotics effective against a **wide range of microorganisms** such as both Gram-Positive and Gram-Negative bacteria = **Broad Spectrum**
- Antibiotics effective mainly against Gram-Positive / Gram-Negative but not both = **Narrow Spectrum**
- Antibiotics effective against a single microorganism = **Limited Spectrum**
- Antibiotic molecules produced by a microbe that are subsequently modified by an organic chemist to enhance their antimicrobial properties or to render them

Bacteria	Antibiotics
<i>Streptomyces sp.</i>	Amphotericin B, Chloramphenicol, Erythromycin, Kanamycin, Neomycin, Nystatin, Rifampin, Streptomycin, Tetracycline
<i>Micromonospora sp.</i>	Gentamicin
<i>Bacillus sp.</i>	Bacitracin, Polymyxin

Mode of action of antibiotics:

Antibiotics inhibit microbial populations by any of 5 major methods:

1. Disruption of cell wall synthesis
2. Interference with cell membrane function
3. Inhibition of protein synthesis or interference with its completion
4. Disruption of functioning of nucleic acids

Cell wall synthesis inhibition	
Antibiotics	Mechanism of action
Penicillin	Inhibit Transpeptidation enzymes involved in the cross-linking
Vancomycin	Binds directly to the D-Ala-terminus and inhibits Transpeptidation
Cell membrane disruption	
Antibiotics	Target
Polymyxine B	Disrupts the structure and permeability of the plasma membrane

Protein synthesis inhibition	
Antibiotic	Target
Puromycin	A site of ribosome
Kanamycin	16S rRNA
Neomycin	16S rRNA
Streptomycin	30 S ribosome
Gentamicin	16S rRNA
Tetracycline	A site of ribosome
Chloramphenicol	Peptidyltransferase
Erythromycin	23S rRNA
Kirromycin	Translation elongation factor Tu

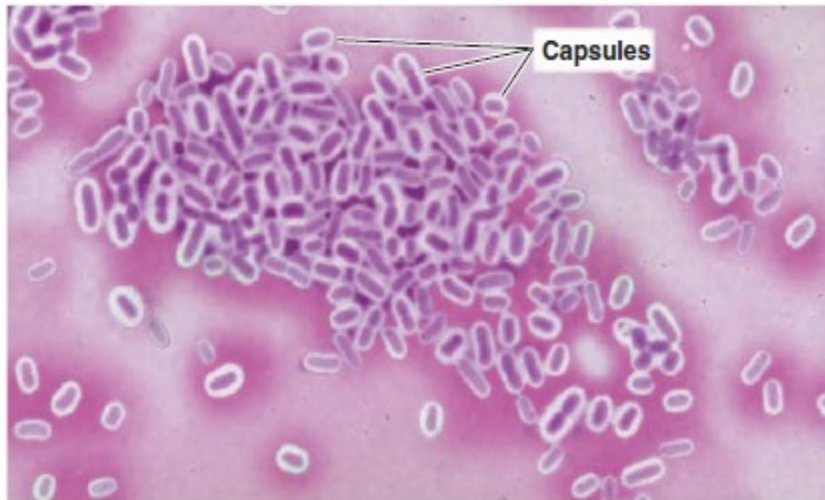
Antibiotics that block replication	
Antibiotics	Target
Ciprofloxacin	Inhibit bacterial DNA gyrase
Hydroxyurea	Ribonucleotide reductase
Nalidixic acid	gyrA subunits of gyrase
Novobiocin	gyrB subunits of gyrase
Mitomycin C	Cross-links DNA

Antibiotics that block RNA synthesis	
Antibiotics	Target
Streptolydigin	Beta subunit of RNA polymerase
Actinomycin D	Binds DNA
Rifampin	Beta subunit of RNA polymerase
Plazomicin	Binds DNA

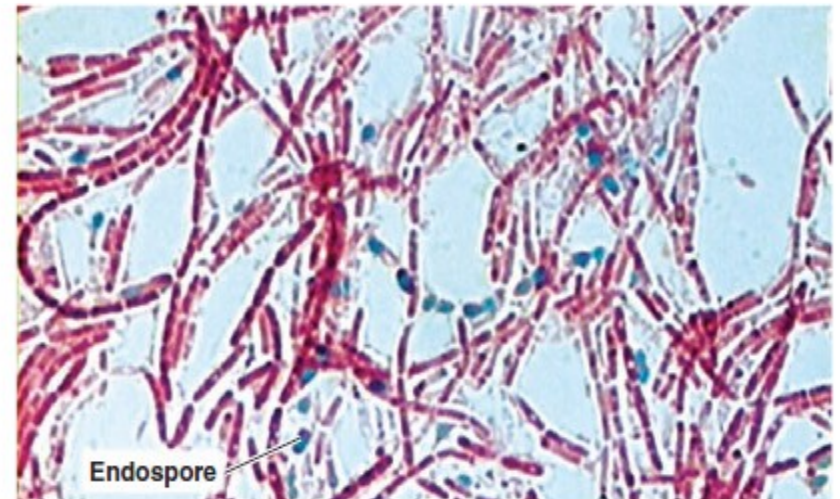
Metabolic antagonism	
Antibiotics	Target
Sulfonamides	Inhibits folic acid synthesis by competition with p-aminobenzoic acid
Trimethoprim	Blocks tetrahydrofolate synthesis
Dapsone	Interferes with folic acid synthesis
Isoniazid	May disrupt pyridoxal or NAD metabolism and functioning

Staining

- A biochemical technique of coloring specimens
- Dyes are used to stain specimens
- **Dyes**- Organic compounds consisting of two functional chemical groups- one group gives dyes its characteristic colour; another group contains an ionizable chemical structure, which helps to solubilize the dye and facilitates its binding to different structures
- Dyes are classified into: **Anionic Dyes** and **Cationic Dyes**
 - **Cationic Dye/ Basic Dye**- Ex. **Crystal Violet, Methylene Blue, Safranin, Malachite Green**
 - React with groups that have a negative charge
 - **Anionic Dye/ Acidic Dye**- Ex. **Eosin, Acid Fuchsin**
 - React with groups that have a positive charge
- **Bacterial staining protocols:**
- 3 basic types- **Simple, Differential** and **Specialized**
- **Simple stains**- React uniformly with all cell types and only distinguishes the organisms from their surroundings
- **Differential stains**- Do not stain all types of cells with the same colour; it discriminates different cell types depending upon the chemical or physical composition of cells
 - The most frequently used staining for bacteria are the **Gram stain** and the **Acid-fast Stain**
- **Specialized stains**- Detect specific structures of cells such as flagella and



(a) Negative staining

LM 10 μ m

(b) Endospore staining

LM 12 μ m

(c) Flagella staining

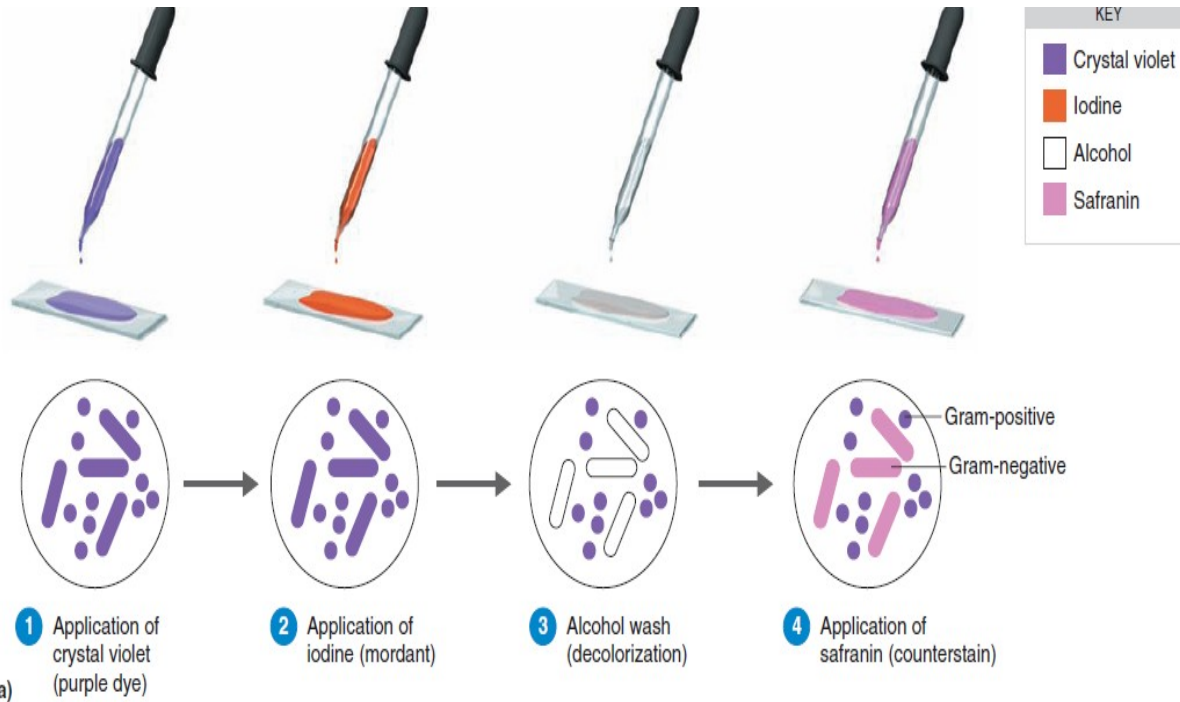
LM 4 μ m

Figure 3.14 Special staining. (a) Capsule staining provides a contrasting background, so the capsules of these bacteria, *Klebsiella pneumoniae*, show up as light areas surrounding the stained cells. (b) Endospores are seen as green ovals in these rod-shaped cells of the bacterium *Bacillus cereus*, using the Schaeffer-Fulton endospore stain. (c) Flagella appear as wavy extensions from the ends of these cells of the bacterium *Spirillum volutans*. In relation to the body of the cell, the flagella are much thicker than normal because layers of the stain have accumulated from treatment of the specimen with a mordant.

Q Of what value are capsules, endospores, and flagella to bacteria?

Gram staining:

- Invented by Danish scientist **Han Christian Gram** in 1884
- Differential staining method for differentiating bacterial species into two groups based on the physical properties of their cell walls



First staining with basic dye-**Crystal Violet** (primary stain)
Imparts purple colour to all cells

Treated with **Gram's Iodine solution** (mordant- used to increase the affinity of the stain for biological specimen)

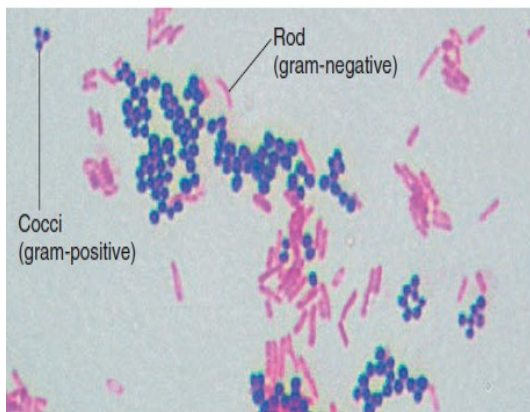
Allows the stain to be retained better by forming an insoluble crystal violet- iodine complex

Addition of **Ethyl Alcohol** And **Acetone** (Gram's decolorizer- removes the stain from the specimen)

This is the differential step, after this step some bacteria retain the purple colour while some other lose purple colour

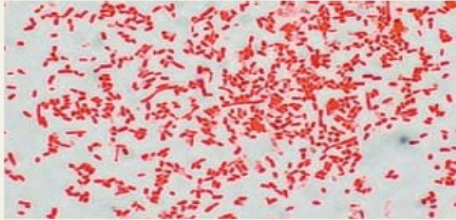
Bacteria that retained colour= **Gram- Positive**
Bacteria that lose purple colour= **Gram-Negative**

- Application of counterstain Safranin (basic dye)
- To make Gram- Negative bacteria visible and becomes directly stained by the **Safranin**
- The Gram- Positive bacteria (which are already stained purple) are not affected by counterstain
- Gram-Negative bacteria



LM 1.5 μm

TABLE 4.1 Some Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

Characteristic	Gram-Positive	Gram-Negative
	 LM 6 μm	 LM 15 μm
Gram Reaction	Retain crystal violet dye and stain blue or purple	Can be decolorized to accept counterstain (safranin) and stain pink or red
Peptidoglycan Layer	Thick (multilayered)	Thin (single-layered)
Teichoic Acids	Present in many	Absent
Periplasmic Space	Absent	Present
Outer Membrane	Absent	Present
Lipopolysaccharide (LPS) Content	Virtually none	High
Lipid and Lipoprotein Content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
Flagellar Structure	2 rings in basal body	4 rings in basal body
Toxins Produced	Exotoxins	Endotoxins and exotoxins
Resistance to Physical Disruption	High	Low
Cell Wall Disruption by Lysozyme	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to Penicillin and Sulfonamide	High	Low
Susceptibility to Streptomycin, Chloramphenicol, and Tetracycline	Low	High
Inhibition by Basic Dyes	High	Low
Susceptibility to Anionic Detergents	High	Low
Resistance to Sodium Azide	High	Low
Resistance to Drying	High	Low

Acid- fast staining

- Type of differential stain
- Used to identify acid-fast organisms such as members of the genus

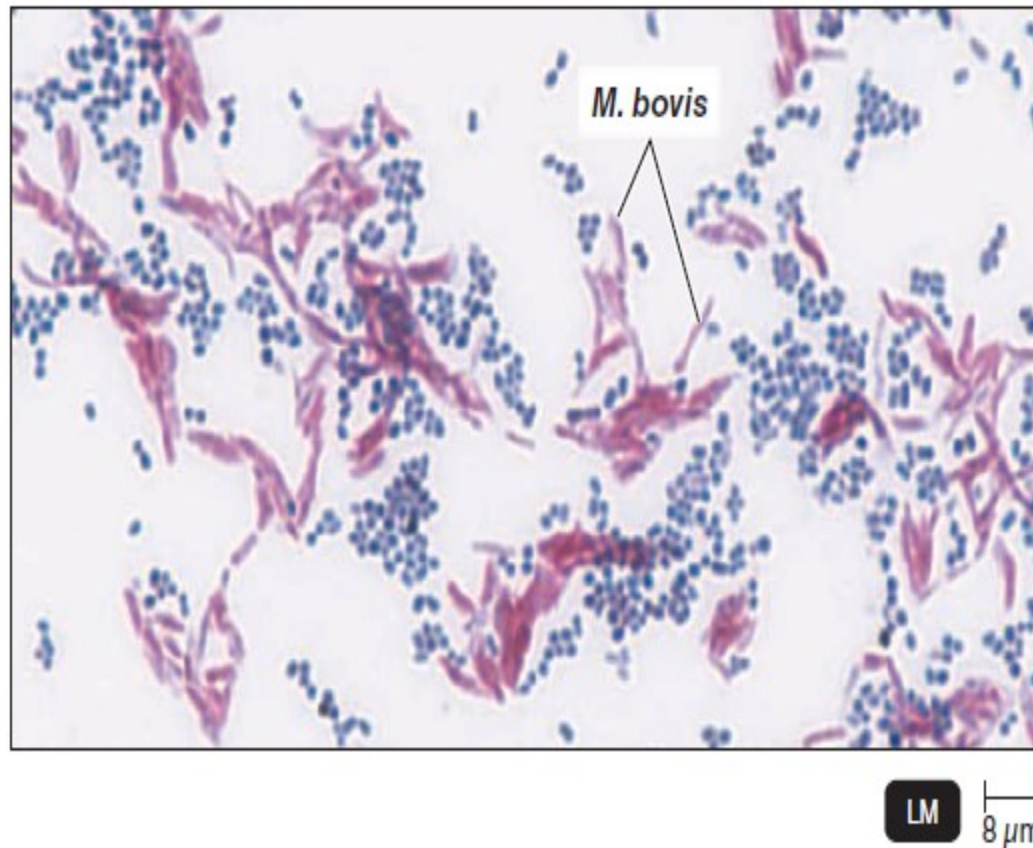


Figure 3.13 Acid-fast bacteria. The *Mycobacterium bovis* bacteria that have infected this tissue have been stained pink or red with an acid-fast stain. Non-acid-fast cells (*Staphylococcus*) are stained with the methylene blue counterstain.

Heating of bacteria with a mixture of basic **Fuchsin** and **Phenol** (also known as **Ziehl-Neelsen Stain**)

Presence of phenol and heat treatment- helps the stain penetrate the cell wall

Once basic Fuchsin has penetrated cell wall, acid-fast cells are not easily decolorized by an **Acid-alcohol Treatment** and hence remain red (due to the presence of

mycolic acid). Non acid-fast bacteria are decolorized by acid-alcohol (because they are colorless after the treatment with decolorizer, they are no longer

visible). Application of counterstain **Methylene Blue** (stains non-acid fast bacteria blue)

TABLE 3.3 A Summary of Various Stains and Their Uses

Stain	Principal Uses
Simple (methylene blue, carbolfuchsin, crystal violet, safranin)	Used to highlight microorganisms to determine cellular shapes and arrangements. Aqueous or alcohol solution of a single basic dye stains cells. (Sometimes a mordant is added to intensify the stain.)
Differential Gram	Used to distinguish different kinds of bacteria. Classifies bacteria into two large groups: gram-positive and gram-negative. Gram-positive bacteria retain the crystal violet stain and appear purple. Gram-negative bacteria do not retain the crystal violet stain; they remain colorless until counterstained with safranin and then appear pink.
Acid-fast	Used to distinguish <i>Mycobacterium</i> species and some species of <i>Nocardia</i> . Acid-fast bacteria, once stained with carbolfuchsin and treated with acid-alcohol, remain pink or red because they retain the carbolfuchsin stain. Non-acid-fast bacteria, when stained and treated the same way and then stained with methylene blue, appear blue because they lose the carbolfuchsin stain and are then able to accept the methylene blue stain.
Special	Used to color and isolate various structures, such as capsules, endospores, and flagella; sometimes used as a diagnostic aid.
Negative	Used to demonstrate the presence of capsules. Because capsules do not accept most stains, the capsules appear as unstained halos around bacterial cells and stand out against a contrasting background.
Endospore	Used to detect the presence of endospores in bacteria. When malachite green is applied to a heat-fixed smear of bacterial cells, the stain penetrates the endospores and stains them green. When safranin (red) is then applied, it stains the remainder of the cells red or pink.
Flagella	Used to demonstrate the presence of flagella. A mordant is used to build up the diameters of flagella until they become visible microscopically when stained with carbolfuchsin.

Bergey's manual of systematic Bacteriology

- In 1923, David Bergey, professor of bacteriology at the university of Pennsylvania and 4 colleagues published a classification of bacteria that could be used for identification of bacterial species, the Bergey's manual of determinative bacteriology
- This manual is now in its 9th edition
- The first edition is more detailed work that contains descriptions of all prokaryotic species currently identified
- Bergey's manual of systematic Bacteriology gives the accepted system of prokaryotic taxonomy
- It is a comprehensive reference work that provides a systematic classification of bacteria and evolutionary relationships
- It covers a wide range of bacterial groups, including **Archaea** and **Eubacteria**
- Provides detailed descriptions of bacterial species, including morphology, metabolism and habitat
- It includes information on bacterial identification, cultivation and preservation
- The latest edition of Bergey's Manual is the 2nd edition, published in multiple volumes



Features of first edition:

- Provides a primarily phenetic classification, and many taxa are not phylogenetically homogeneous
- Easily determined features such as cell shape, gram-staining characteristics, oxygen relationships, motility and the mode of energy production are used to

Features of second edition:

- Phylogenetic method of classification and distributes prokaryotes between two domains and 25 phyla
- Comparisons of nucleic acid sequences, particularly 16S rRNA sequences are the foundation of this

The second edition has 5 volumes:

- **Volume 1: The archaea and the deeply branching and phototropic bacteria**
 - This volume describes the *Archaea*, *Cyanobacteria*, *Green sulfur* and *Nonsulfur* bacteria, *Deinococci* and other deeply branching groups
- **Volume 2: The proteobacteria**
 - All of the proteobacteria (purple bacteria) are placed in this volume and are divided into 5 groups based on rRNA sequences and other characteristics: *α -proteobacteria*, *β -proteobacteria*, *γ -proteobacteria*, *δ -proteobacteria* and *ϵ -proteobacteria*
- **Volume 3: The low G+C Gram- Positive bacteria**
 - This volume contains Gram- Positive bacteria with G+C content below 50%
 - Some of the major groups are the *Clostridia*, *Bacilli*, *Streptococci* and *Staphylococci*
 - *Mycoplasma* are also placed here
- **Volume 4: The high G+C Gram-Positive bacteria**
 - Gram- Positive bacteria with G+C content above around 50-55% are in this volume
 - Such groups as *Corynebacterium*, *Mycobacterium*, *Nocardia* and the *Actinomycetes* are located here

Volume 1: ***The Archaea and the Deeply Branching and Phototrophic Bacteria***

Volume 2: ***The Proteobacteria***

Domain Archaea

Domain Bacteria

Phylum
Crenarchaeota

Phylum
Euryarchaeota

Class I. Methanobacteria
Class II. Methanococci
Class III. Halobacteria
Class IV. Thermoplasmata
Class V. Thermococci
Class VI. Archaeoglobi
Class VII. Methanopyri

Phylum Aquificae
Phylum Thermotogae
Phylum
Thermodesulfobacteria
Phylum "Deinococcus-
Thermus"
Phylum Chrysiogenetes
Phylum Chloroflexi
Phylum Thermomicrobia
Phylum Nitrospira
Phylum Deferribacteres
Phylum Cyanobacteria
Phylum Chlorobi

Phylum Proteobacteria

Class I.
Alphaproteobacteria
Class II. Betaproteobacteria
Class
III. Gammaproteobacteria
Class IV.
Deltaproteobacteria

Volume 3: ***The Low G C Gram-Positive Bacteria***

Phylum
Firmicutes

Class I. Clostridia
Class II. Mollicutes
Class III. Bacilli

Volume 4: ***The High G C Gram-Positive Bacteria***

Phylum Actinobacteria

Class
Actinobacteria

Volume 5. ***The Planctomycetes, Spirochaetes, Fibrobacteres, Bacteroidetes, and Fusobacteria***

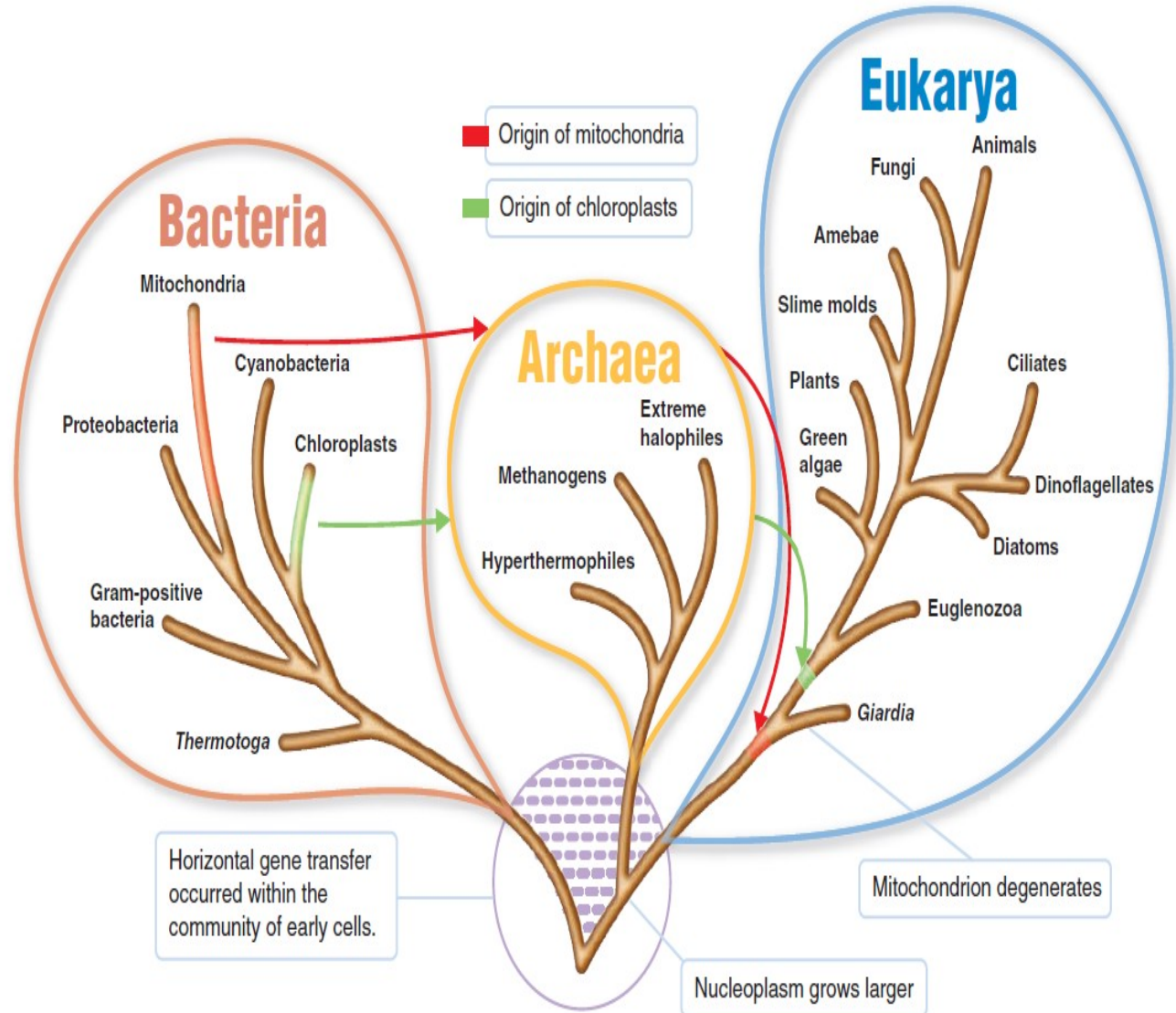
Phylum Planctomycetes
Phylum Chlamydiae
Phylum Spirochaetes
Phylum Fibrobacteres
Phylum Acidobacteria
Phylum Bacteroidetes
Phylum Fusobacteria
Phylum Verrucomicrobia
Phylum Dictyoglomi

The Three-Domain System



Carl R. Woese's 3 domain classification of bacteria

- In 1990 Carl R. Woese and his collaborators proposed a new classification system that divides life forms into 3 domains
- They have used rRNA studies to group all living organisms into three domains



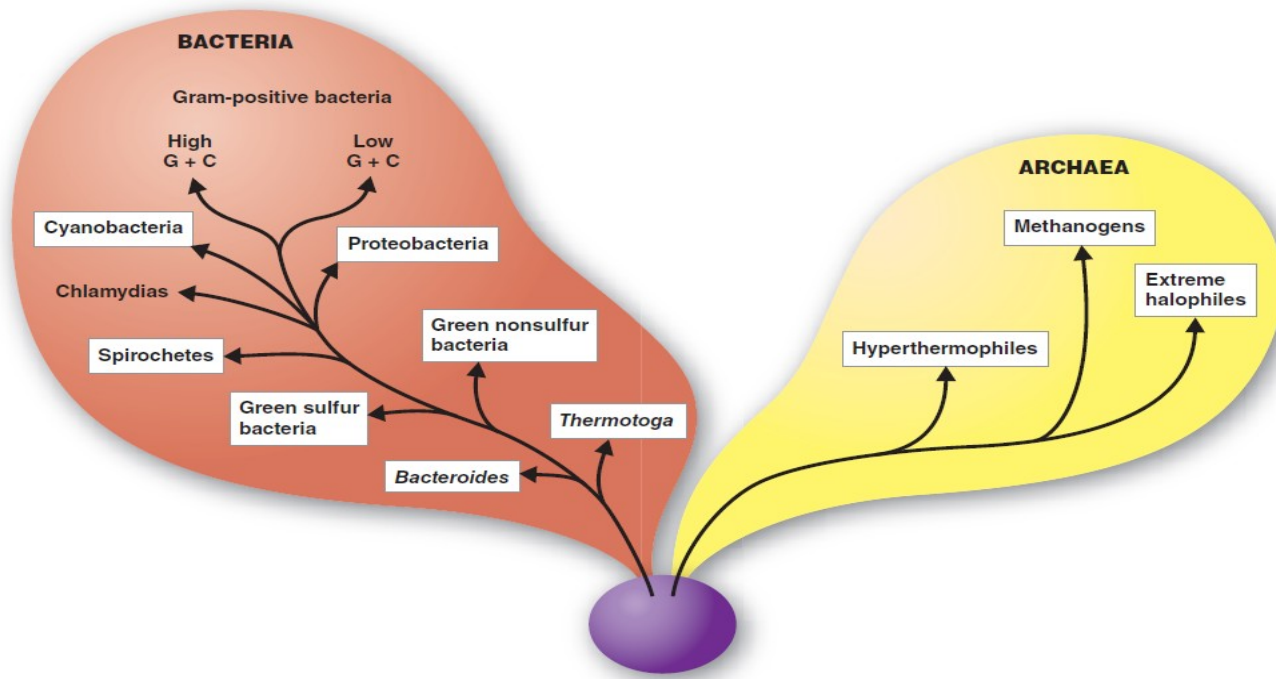
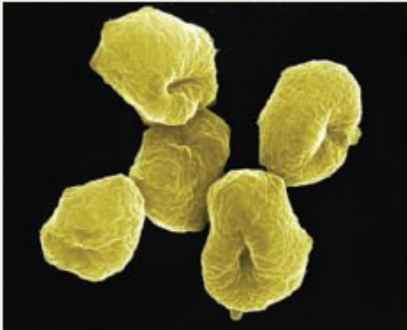
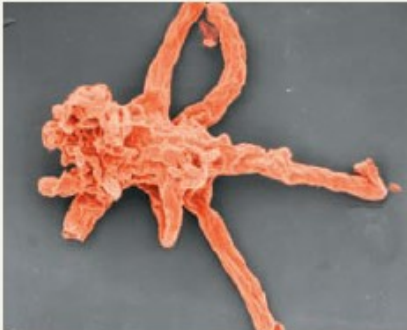


Figure 10.6 Phylogenetic relationships of prokaryotes. Arrows indicate major lines of descent of bacterial groups. Selected phyla are indicated by the white boxes.

- Thus there are two quite different groups of prokaryotes- **The Bacteria** and **The Archaea**
- **The Bacteria-** includes vast majority of prokaryotes
- Among other properties, bacteria either have cell wall Peptidoglycan containing muramic acid or are related to bacteria with such cell walls and have membrane lipids with ester-linked, straight- chained fatty acids that resemble eukaryotic membrane lipids
- **The Archaea-** includes groups which differ from bacteria in many respects and resemble eukaryotes in some ways
- They differ from bacteria in lacking muramic acid in their cell walls and possessing membrane lipids with ether- linked branched aliphatic chains. transfer

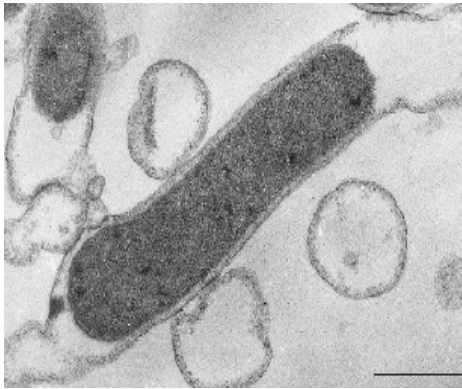
TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

	Archaea	Bacteria	Eukarya
	 <i>Sulfolobus</i> SEM 1 μm	 <i>E. coli</i> SEM 1 μm	 <i>Amoeba</i> SEM 5 μm
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine	Methionine
Antibiotic Sensitivity	No	Yes	No
rRNA Loop*	Lacking	Present	Lacking
Common Arm of tRNA [†]	Lacking	Present	Present

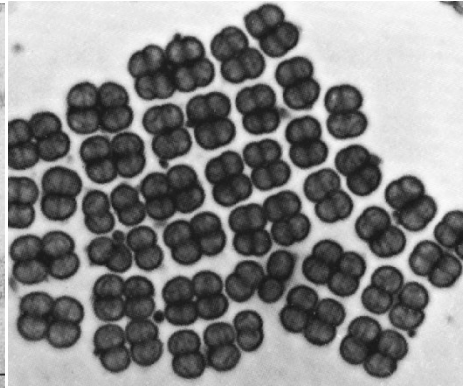
*Binds to ribosomal protein; found in all bacteria.
[†]A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

General features of **Bacteria** domain:

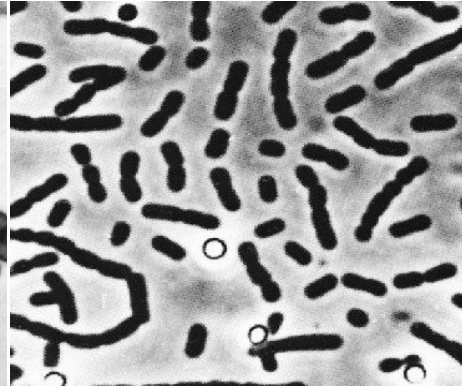
Membrane-enclosed nucleus with nucleolus	Absent
Complex internal membranous organelles	Absent
Cell wall	Almost have Peptidoglycan containing muramic acid
Membrane lipid	Have ester-linked, straight-chained fatty acids
Gas vesicles	Present
Transfer RNA	Thymine present in most tRNAs N- formylmethionine carried by initiator tRNA
Polycistronic mRNA	Present
mRNA introns, mRNA splicing, Capping, Poly A tailing	Absent
Ribosomes	70S; elongation factor 2- does not react with diphtheria toxin; sensitive to Chloramphenicol and kanamycin; insensitive to anisomycin
DNA dependent RNA polymerase	One; sensitive to rifampicin
Polymerase II type promoters	Absent
Metabolism	Similar ATPase, no methanogenesis



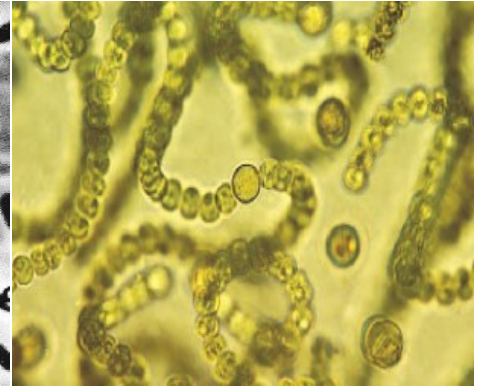
Thermotoga maritima



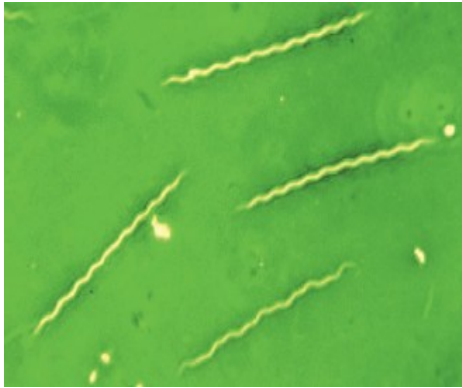
Deinococcus radiodurans



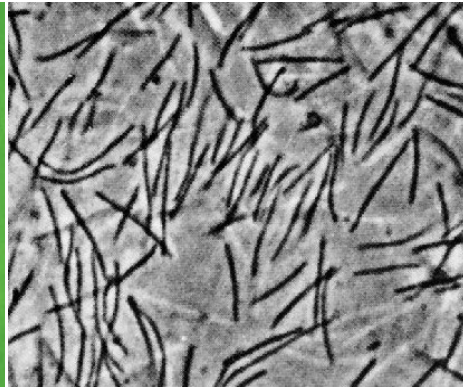
**Green Sulfur Bacteria-
*Chlorobium limicola***



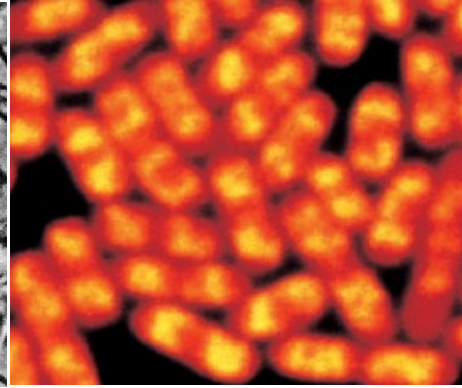
**Oxygenic
Photosynthetic**



Spirochetes-Treponema pallidum



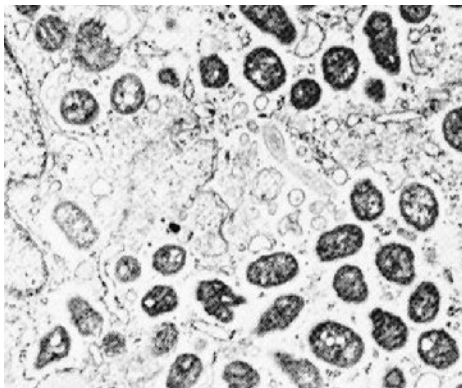
**Nonphotosynthetic,
Bacteria-**



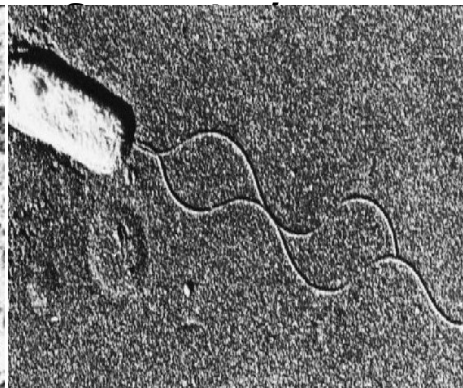
Salmonella typhi



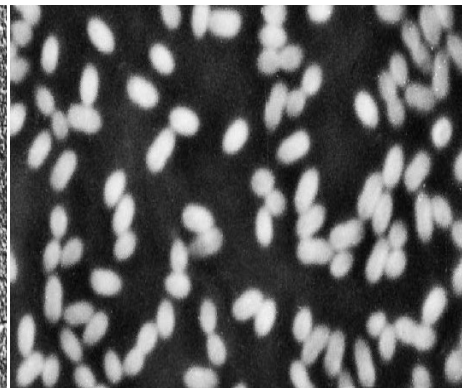
Rhodospirillum rubrum



Rickettsia sp.



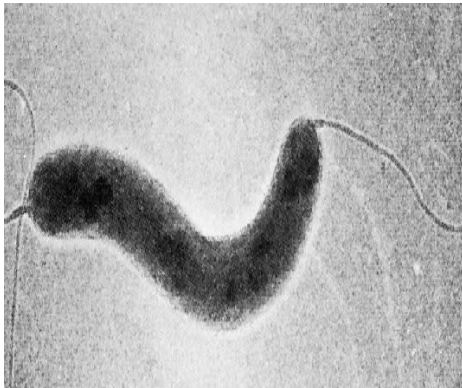
Rhizobium leguminosarum



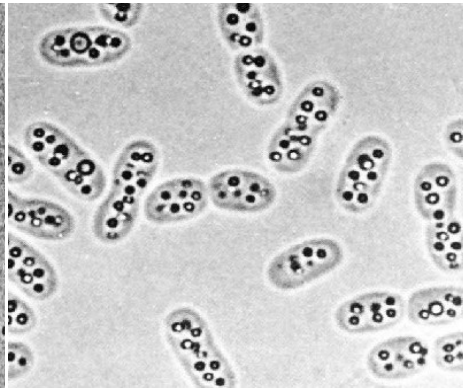
Nitrosomonas europaea



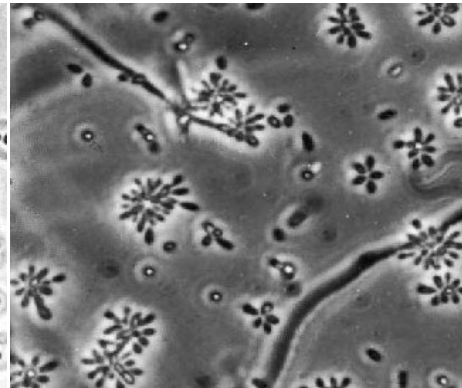
Spirillum volutans



Colorless Sulfur Bacteria-



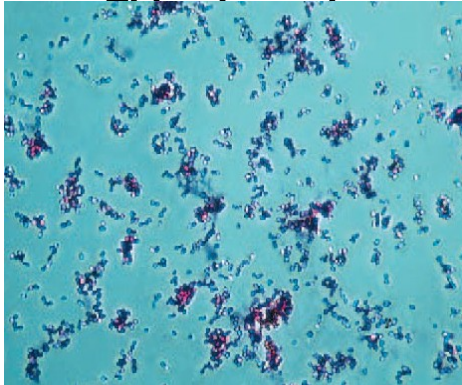
**Purple Sulfur Bacteria -
Chromatium vinosum**



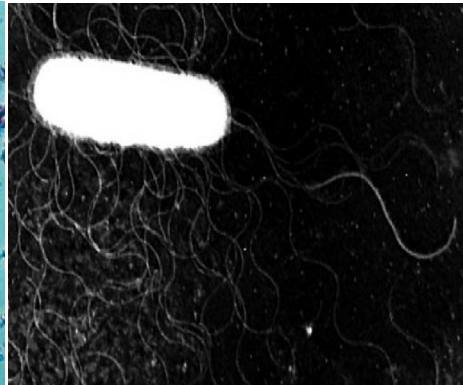
Leucothrix mucor



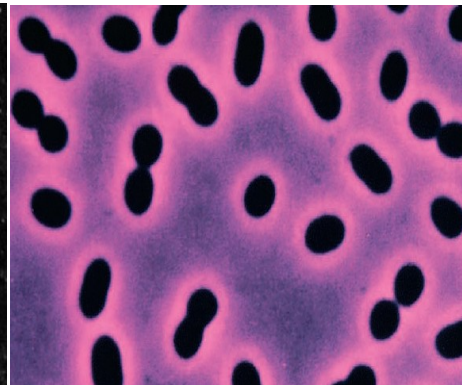
Pseudomonas putida



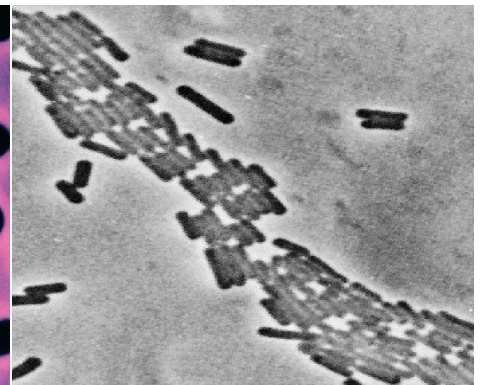
Azotobacter



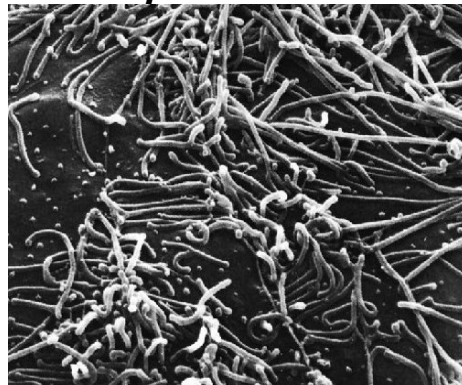
Vibrio alginolyticus



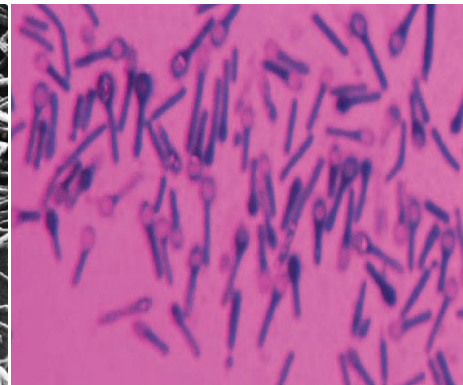
Desulfobacter



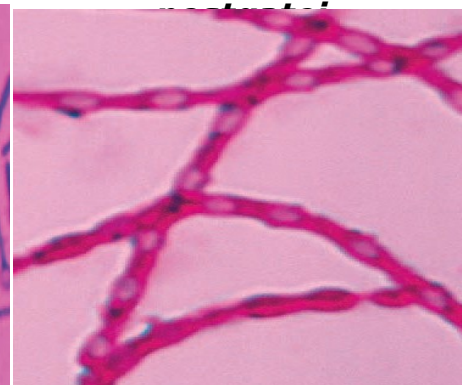
Chondromyces crocatus



Mycoplasma pneumoniae



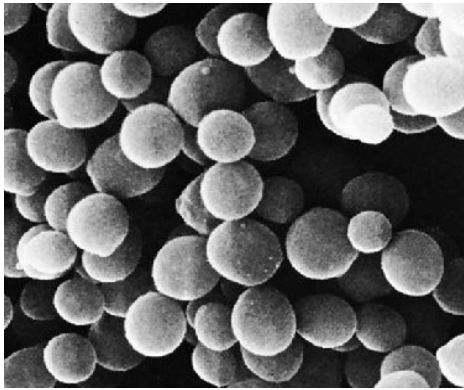
Clostridia tetani



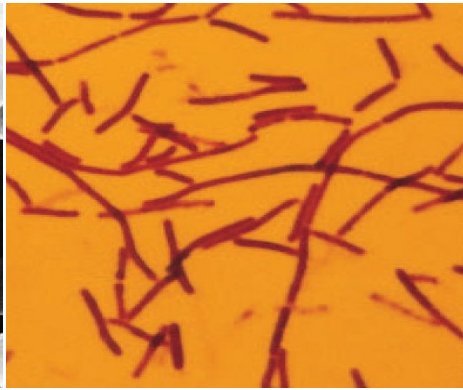
Bacillus subtilis



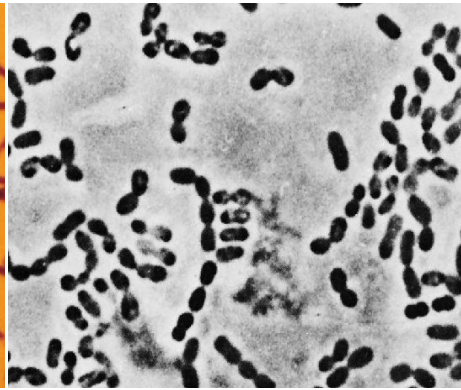
Thermoactinomyces



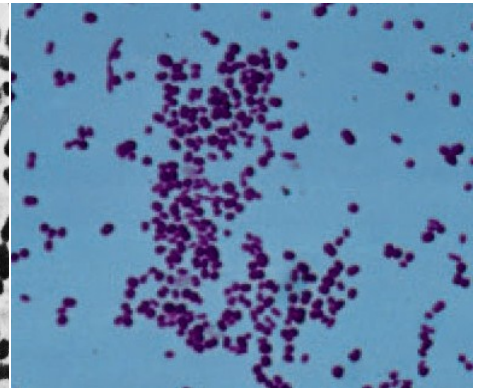
Staphylococcus aureus



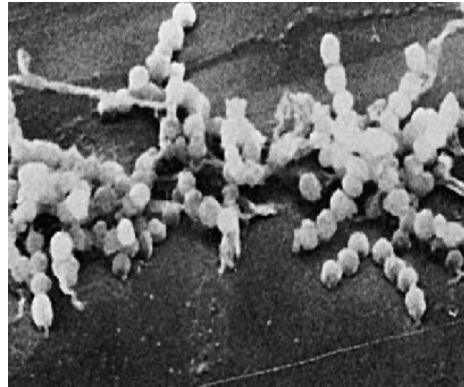
Lactobacillus



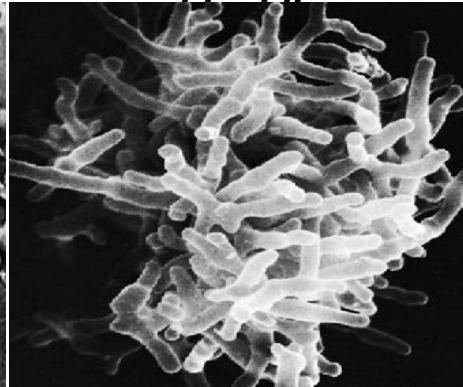
Leuconostoc



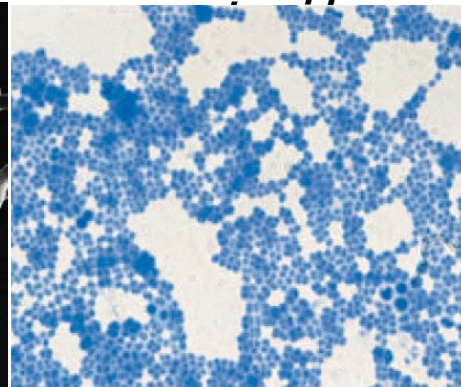
Streptococcus



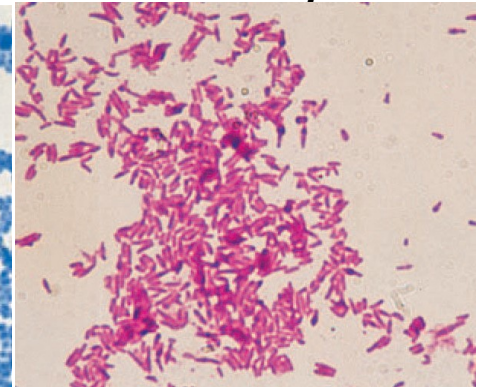
Saccharopolyspora sp.



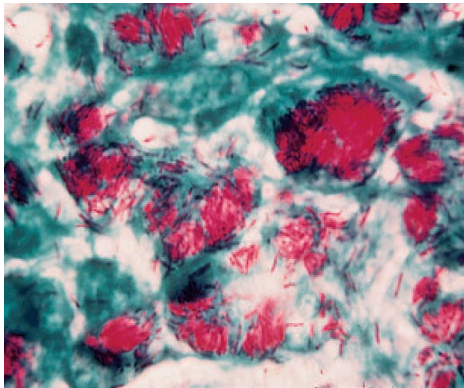
Actinomyces sp.



Micrococcus luteus



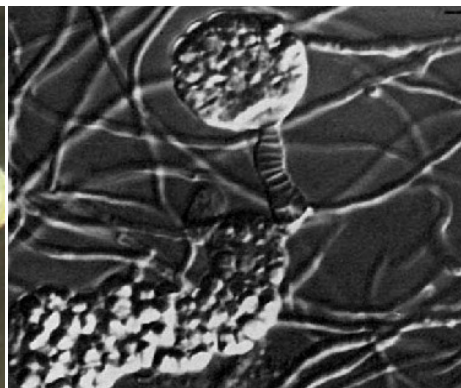
Corynebacterium



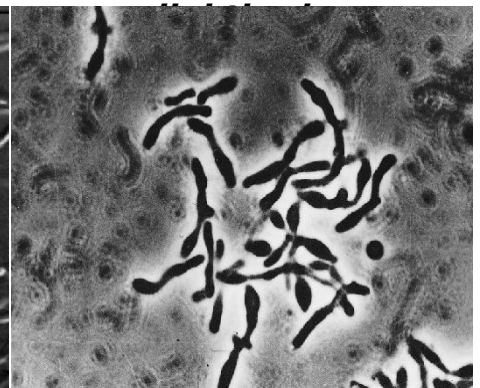
Mycobacterium leprae



Streptomyces griseus



Frankia sp.



***Bifidobacterium
bifidum***

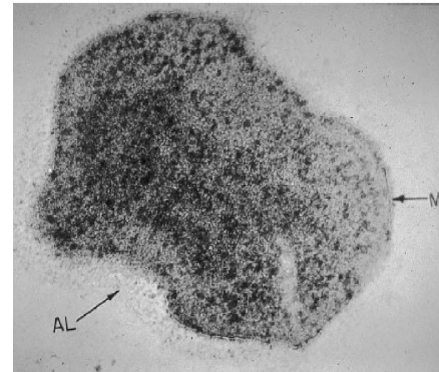
General features of **Archaea** domain:

Membrane-enclosed nucleus with nucleolus	Absent
Complex internal membranous organelles	Absent
Cell wall	Various types, no muramic acid
Membrane lipid	Have ether- linked, branched aliphatic chains
Gas vesicles	Present
Transfer RNA	No thymine in T or T ψ C arm of tRNA methionine carried by initiator tRNA
Polycistronic mRNA	Present
mRNA introns, mRNA splicing, Capping, Poly A tailing	Absent
Ribosomes	Size- 70S; Elongation factor 2 reacts with diphtheria toxin; insensitive to Chloramphenicol and kanamycin; sensitive to anisomycin
DNA dependent RNA polymerase	Several; insensitive to rifampicin
Polymerase II type promoters	Present
Metabolism	Similar ATPase- yes; methanogenesis- present; nitrogen fixation- present; chlorophyll based photosynthesis- absent

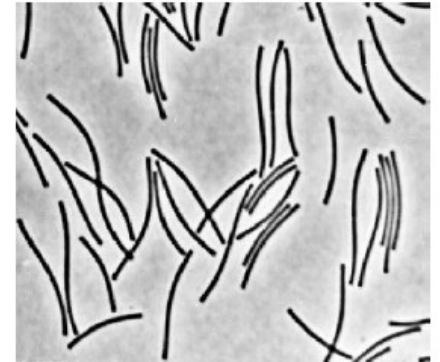


Habitat of Archaea

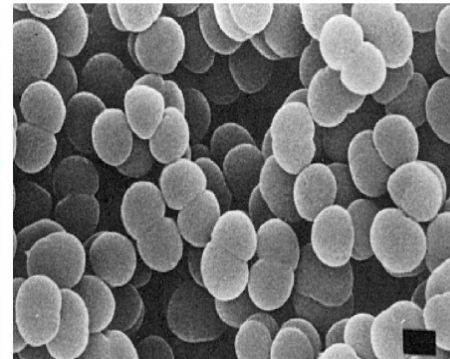
Ex. The pump geyser in Yellowstone National Park. The orange color is due to the carotenoid pigments of the microorganisms.



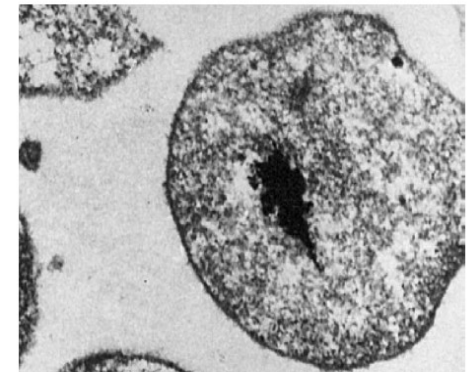
Sulfolobus brierleyi



Bacillus anthracis



Halococcus morrhuae



Thermoplasma

General features of **Eukarya** domain:

Membrane-enclosed nucleus with nucleolus	Present
Complex internal membranous organelles	Present
Cell wall	no muramic acid
Membrane lipid	Have ester-linked, straight-chained fatty acids
Gas vesicles	Absent
Transfer RNA	Thymine present methionine carried by initiator tRNA
Polycistronic mRNA	absent
mRNA introns, mRNA splicing, Capping, Poly A tailing	present
Ribosomes	Size- 80S (cytoplasmic ribosomes); Elongation factor 2 reacts with diphtheria toxin; insensitive to Chloramphenicol and kanamycin; sensitive to anisomycin
DNA dependent RNA polymerase	3; insensitive to rifampicin
Polymerase II type promoters	Present
Metabolism	Similar ATPase- yes; methanogenesis-absent; nitrogen fixation-absent; chlorophyll-based photosynthesis-present ^a ;

References:

1. Gerard J. Tortora, Berdell R. Funke, Christine L. Case(2013), Microbiology- An Introduction, Pearson Education Inc, 11th edition, Page no.75-241
2. Lansing M Prescott (2002), Microbiology, The McGraw-Hill Companies, 5th edition, Page no. 14-149, 430-560
3. <https://www.aajjo.com/product/milk-pasteurization-machine>
4. <https://www.anyrgb.com/en-clipart-slqiu>

